CHARACTERIZATION OF TRICHOMES IN *LENS* SPP. AND THEIR EFFECT ON DROUGHT RESISTANCE, HERBICIDE EFFICACY, AND PEA APHID FECUNDITY

A Thesis Submitted to the
College of Graduate and Postdoctoral Studies
In Partial Fulfillment of the Requirements
For the Degree of Master of Science
In the Department of Plant Sciences
University of Saskatchewan
Saskatoon

By

ISHITA PATEL

© Copyright Ishita Patel, June 2020. All rights reserved.

Permission to Use

In presenting this thesis in partial fulfillment of the requirements for a graduate degree from the University of Saskatchewan, I agree that the Libraries of this University may make it freely available for inspection. I further agree that permission for copying of this thesis in any manner, in whole or in part, for scholarly purposes may be granted by the professor or professors who supervised my thesis work or, in their absence, by the Head of the Department or the Dean of the College in which my thesis work was done. It is understood that any copying or publication or use of this thesis or parts thereof for financial gain shall not be allowed without my written permission. It is also understood that due recognition shall be given to me and to the University of Saskatchewan in any scholarly use which may be made of any material in my thesis.

Requests for permission to copy or to make other uses of materials in this thesis in whole or part should be addressed to:

Head of the Department of Plant Sciences
College of Agriculture and Bioresources
University of Saskatchewan
51 Campus Drive
Saskatoon, Saskatchewan S7N 5A8 Canada

OR

Dean
College of Graduate and Postdoctoral Studies
University of Saskatchewan
116 Thorvaldson Building, 110 Science Place
Saskatoon, Saskatchewan S7N 5C9 Canada

Abstract

Drought, weeds, and increasing insect pressure are imminent threats to lentil production in the Canadian prairies. This research characterized trichomes (surface hairs) in wild and cultivated lentil and explored their role in imparting resistance to drought, herbicides, and the pea aphid.

In a growth chamber study, 20 wild and cultivated lentil genotypes from seven species were subjected to fully watered and moderate drought conditions. Microstructures on adaxial leaf surfaces were characterized and transpiration rate in 12 genotypes across all species was determined. Drought response of trichomes across species was inconsistent and differed with genotype: While some genotypes increased their trichomes under drought, others decreased them. Similar results were observed upon measuring traits of trichome length, epidermal cell density, and stomatal index. Among the 12 genotypes in which transpiration rate was determined, most genotypes reduced transpiration under drought and this decrease was associated with an increase in trichome density. However, some genotypes responded to drought by increasing transpiration and reducing trichomes, indicating that response to drought is unique to each genotype and other mechanisms are responsible for drought tolerance in lentil.

Greenhouse experiments were conducted to test trichome influence on glyphosate tolerance and spray droplet retention using water and water + non-ionic surfactant solution. A set of recombinant inbred lines using *L. culinaris* CDC Redberry and *L. tomentosus* IG 72805 as parents were selected based on varying trichome characteristics on adaxial leaf surfaces. While glyphosate tolerance studies proved inconclusive, surface spray retention decreased with increasing trichome density upon addition of non-ionic surfactant. Results indicate that trichomes might improve herbicide resistance by preventing surface droplet retention.

Lastly, pea aphid performance was monitored on lentil cultivars CDC Redberry and CDC Maxim, and *L. tom.* IG 72805, which exhibit low, intermediate, and high trichome density on adaxial leaf surfaces, respectively. Pea aphids had the least mortality, largest adult size and least maturity time on *L. tom.* IG 72805. Their biosis was lowest on CDC Maxim and intermediate on CDC Redberry, suggesting that cultivated lentil has antibiosis potential.

Altogether, this research shows that the role of trichomes in imparting drought resistance in lentil is complex and genotype dependent. While trichomes are not beneficial in imparting pea aphid resistance, they might help in the development of herbicide tolerant lentil cultivars.

Acknowledgements

I would like to express my deepest gratitude to my supervisor, Albert Vandenberg. Not only was he incredibly supportive throughout my research, he also encouraged me to pursue my interests outside of academics which greatly enriched my graduate school experience. I am immensely grateful to my committee members: Karen Tanino, Sean Prager, and Eric Lamb for providing their expert advice on topics I was initially unfamiliar with and for being ever so helpful and patient. Thanks to my external examiner, Lyndon Porter, for his thought-provoking questions that contributed to improving this thesis.

A huge thanks is extended to Linda Gorim, who took me under her wings the moment I got to the U of S and gave me technical as well as personal advice. Appreciation is also extended to Ning, Dilanganie, Devini, Brent, Donna, Kevin, Dayna, as well as the phytotron staff and the Pulse Lab field crew for their help with experimental setup, troubleshooting, and data collection. Aspects of this work would not have been completed without technical help from Akiko, Rob, Monica, Guosheng, and Sue Abrams and her group. I am grateful to them for their patience in teaching me experimental techniques and for providing valuable input.

I would like to acknowledge the financial support provided by Robert P. Knowles scholarship, Robert R. Moffatt scholarship, the NSERC CGS-M scholarship, and Michael Smith Foreign Study Supplement through which I completed a part of my project at the University of Wyoming. Over at the University of Wyoming, I am grateful to Andrew Kniss and his group for a great collaborative experience.

During this thesis I have had the pleasure of meeting and interacting with some truly wonderful people who always cheered me on and were there for me whenever I needed help. To them and to all my friends, old and new, thank you for your unwavering support.

I would like to also thank all my past mentors, advisors, family friends and teachers who encouraged me to pursue Science growing up. You were instrumental in shaping my academic career and I would not be here had you not planted those seeds and watered them.

Finally, I am incredibly grateful to my parents for their unconditional love, understanding, and encouragement. A special thanks to my sister, Tushita, for being a great source of rational advice and moral support. Also, because she really wanted to be mentioned in this thesis.

Table of Contents

List of Tables

List of Figures

List of Appendices

List of Abbreviations

ABA	Abscisic acid
AgBio	Agriculture and Bioresources building
ALS	Acetolactate synthase enzyme
ANOVA	Analysis of Variance
CDC	Crop Development Centre
CPWC	Critical period of weed control
DAP	Days after planting of pre-germinated seeds
DAS	Days after seeding without pre-germinating the seeds
DAT	Days after treatment
df	Degrees of freedom
ED_{50}	Dose of herbicide eliciting 50% response
FAO	Food and Agriculture Organization of the United Nations
FC	Field capacity
FW	Fully watered
GLM	Generalized linear model
L. cul.	*Lens culinaris*
L. erv.	*Lens ervoides*
L. lam.	*Lens lamottei*
L. nig.	*Lens nigricans*
L. ode.	*Lens odemensis*
L. ori.	*Lens orientalis*
L. tom.	*Lens tomentosus*
MRL	Maximum residue limit of pesticide in seed
SUMP	Suzuki's Universal Micro-Printing
U of S	University of Saskatchewan

Chapter 1
Introduction

Lentil (*Lens culinaris* Medik.) is an important crop of the agriculture industry of Saskatchewan. Canada is the world's largest lentil producer and exporter, and about 90% of Canada's lentils are produced in Saskatchewan (Agriculture and Agri-Food Canada, 2019). Lentil is an environmentally beneficial cash crop as it helps diversify crop rotations while requiring less nitrogen fertilizer input due to its ability to fix nitrogen via root nodules (Warne et al., 2019). Lentils are a highly nutritious source of protein, vitamins, fibre, and carbohydrates. They are also a source of many essential macro and micro-nutrients including phosphorous, potassium, magnesium, calcium, sodium, iron, zinc, copper, and manganese (Shahwar et al., 2017).

Lentils are consumed as a whole food in more than 100 countries. With global population predicted to rise to 9.8 billion by 2050, there will be increased demand for food per capita in the future (Bruinsma, 2009). The need for more food will increase land and water use for agriculture, and increased competition for natural resources. Increased industrial and urban developmental domains will further complicate the situation (Postel, 2000). Moreover, with predictions of changes in climate and precipitation patterns in the Canadian prairies, the occurrence of drought is expected to increase in frequency and intensity (Government of Canada, 2008). Elevated carbon dioxide levels in the atmosphere have also been found to modify plant-insect interactions, positively affecting the feeding efficiency and fecundity of pea aphid, a major legume pest in Western Canada (Sun et al., 2016).

Lentil is tolerant to a limited number of herbicides, and its poor ability to compete with weeds leads to severe yield loss. Developing new and improved strategies for weed control is essential in lentil because there are few herbicide options for post emergent use in lentil and weeds are increasingly becoming resistant to herbicides (Heap, 2020; McDonald et al., 2007; Smitchger et al., 2012). Thus, to ensure global food security as well as to maintain good economic returns to producers in Canada, there is a critical need to identify important traits and to develop lentil germplasm that is herbicide tolerant and can maintain sustainable yield in the wake of increased water deficits and pest populations.

With respect to sustenance during drought, various mechanisms to adapt to water deficit exist in legumes. These include changing source-sink dynamics to partition more nutrients towards

reproductive structures that lead to delayed or early flowering to escape drought, developing a deep root system, and developing morphological traits such as thickened leaf cuticles and development of pubescence (trichomes) with reduced leaf size to limit water loss (Graham and Vance, 2003). Most of these strategies have been observed in lentil, and wild lentil genotypes originating from the drought prone areas of the Middle East and the Mediterranean basin have variation in root systems and transpiration rates in comparison to cultivated lentil genotypes (Gorim and Vandenberg, 2017, 2018). Wild lentil genotypes also have morphological variation with respect to trichomes and leaf size, although literature is scarce in this area.

Various mechanisms exist in plants to defend themselves against herbivorous insects - these may be direct, indirect, mechanical or chemical (War et al., 2012). Creating a mechanical or physical barrier on the surface constitutes the first line of defense, which includes leaf surface wax, setae, spines, and trichomes originating from the epidermal layer (Hanley et al., 2007). Surface trichomes may also secrete volatile secondary metabolites and other compounds that help deter insects either directly or indirectly (Fürstenberg-Hägg et al., 2013; War et al., 2012). Legumes such as pigeon pea (*Cajanus cajan*) and chickpea (*Cicer arietinum*) have a high amount of anti-nutritional chemicals like tannin and polyphenols and secrete acidic compounds that give them resistance to insect pests (Sharma et al., 2009; Yoshida et al., 1997). Little is known about the influence of lentil trichomes on insects, or whether insect growth and development differ on wild vs. cultivated lentil.

Physical barriers such as surface wax and presence of trichomes might also protect plants against herbicides by reducing surface wettability and thus limiting the amount of herbicide residue that penetrates the plant (Chachalis et al., 2001; Hess and Falk, 1990). However, herbicide efficacy might also be increased in some species due to the presence of specialized trichomes that provide a site of entry and reduction in contact angle of the sprayed herbicide, subsequently leading to increased absorption of herbicide droplets (Benzing and Burt, 1970; Boutin et al., 2012; Wyrill and Burnside, 1976). Non-ionic surfactants are also commonly added to herbicide formulations to overcome physical barriers posed by plant surfaces. Non-ionic surfactants reduce surface tension of herbicide droplets and increase their spreadability and penetration into the foliar surface, thus increasing herbicide efficacy (Tominack and Tominack, 2000). The influence of trichomes on herbicide efficacy or the effect of the addition of non-ionic surfactant on surface spray retention has not yet been explored in lentil.

This project focuses on pubescence in lentil and aims to characterize the natural variation present in trichomes and other leaf microstructures in wild and cultivated lentil genotypes, and elucidate the potential role played by lentil trichomes in drought tolerance, insect resistance, and herbicide resistance. Ultimately, this research is intended to determine if trichomes have potential value as a trait in lentil breeding and production. The following hypotheses were established at the beginning of the project:

Hypothesis 1. Trichome characteristics vary between *Lens* spp. under fully watered condition, with cultivated lentil having reduced trichome density compared to wild lentil genotypes.

Hypothesis 2. Lentil genotypes grown under drought have increased trichome density and reduced transpiration rate.

Hypothesis 3. Lentil genotypes with higher trichome density and coverage are more resistant to herbicides and have reduced droplet retention on their surface.

Hypothesis 4. Addition of non-ionic surfactant increases droplet retention irrespective of trichome density and coverage.

Hypothesis 5. Increased trichome density in lentil negatively affects pea aphid biosis.

Testing of these hypotheses was carried out by designing experiments with the research objectives described below.

- Characterize trichome density, trichome length, epidermal cell density, and stomatal index on the adaxial leaf surface of twenty selected genotypes within seven *Lens* spp. by measuring and comparing these parameters for plants grown under fully watered and moderate drought conditions (Hypothesis 1)
- Determine the difference in transpiration rate of twelve selected genotypes within seven wild and cultivated *Lens* spp. grown under fully watered and drought condition and relate it to differences observed in surface microstructures (Hypothesis 2)
- Investigate the role of trichomes in influencing glyphosate resistance in lentil and explore how spray droplet retention is affected by the addition of non-ionic surfactant (Hypotheses 3 and 4)
- Determine if pea aphid growth and development is impeded on pubescent wild lentil species *L. tom.* IG 72805 compared to less pubescent lentil cultivars CDC Redberry and CDC Maxim (Hypothesis 5)

Twenty lentil genotypes belonging to 7 *Lens* spp. were used to characterize trichomes and explore their role in drought tolerance and the experiment was conducted under controlled conditions in the U of S AgBio phytotron facility in Saskatoon, Saskatchewan. Studies exploring the role of trichomes in herbicide resistance were performed in greenhouses at the University of Wyoming in Laramie, Wyoming using *L. cul.* CDC Redberry, *L. tom.* IG 72805 and F_5-F_7 derived lines from interspecific NAM 38 (*L. cul.* CDC Redberry x *L. tom.* IG 72805) population. Lastly, the experiment investigating pea aphid biosis was done using cultivars CDC Redberry and CDC Maxim, and wild lentil genotype *L. tom.* IG 72805. This experiment was conducted in a growth chamber under controlled conditions in Saskatoon, Saskatchewan.

Chapter 2
Review of Literature

2.1 Lentil origin, commercial cultivation, and classification into gene pools

Lentil (*Lens culinaris* Medikus) is among the earliest domesticated crops in the Old World (Sandhu and Singh, 2007). Earliest records of lentil cultivation date back to 8500-600 BC in the Turkey-Syria-Iraq region and from there, cultivation spread to Europe, Asia, Northern Africa, then later to first South America and then North America (Laskar et al., 2019; Sandhu and Singh, 2007). Currently, major lentil producers include Canada, India, USA, Turkey, Australia, Kazakhstan, Nepal, Russian Federation, Bangladesh, China, Ethiopia and Syria (FAO, 2018). Cultivated lentil germplasm has been classified into three major groups that correlates with its geographical origins: (a) South Asia (sub-tropical savannah), (b) Mediterranean, and (c) northern temperate (Khazaei et al., 2016). *Lens culinaris* subsp. *orientalis* (Boiss.) Ponert is the presumed wild progenitor of cultivated lentil and is found in Turkey, Syria, Lebanon, Israel, Jordan, Iraq, Iran, Afghanistan, Greece, and Uzbekistan (Ladizinsky, 1979, 1993; Sandhu and Singh, 2007; Zohary, 1972).

Commercial lentil production in North America commenced in late 1930s in eastern Washington and northern Idaho (USA) and in Canada, lentil production began in 1969 (Nleya et al., 2004). Since then, Canada quickly increased its lentil production and is currently the largest producer and exporter of lentil in the world (FAO, 2017, 2018). In Canada, lentil is primarily grown in Saskatchewan where in 2019, it accounted for 92% of total lentil production, and in Alberta, which accounted for 8% of production (Statistics Canada, 2019). Lentil is classified into two major market classes based on colour: red (based on cotyledon colour) and green (based on seed coat colour) (Stefaniak and McPhee, 2015). Commercially, lentil is grouped into four size classes - extra small (less than 30 mg seed weight), small, medium, and large (typically more than 60 mg seed weight). The main export markets for red lentil are in the Middle East and South Asia. Green lentil is graded as small, medium, and large and is mostly exported to markets in Colombia and Western Europe (Stefaniak and McPhee, 2015).

The genus *Lens* consists of seven taxa (Ferguson et al., 2000) based on morphological approaches in classical taxonomy:

L. culinaris Medikus

subsp. *culinaris*

subsp. *orientalis* (Boiss.) Ponert

subsp. *tomentosus* (Ladizinsky) Ferguson, Maxted, van Slageren & Robertson

subsp. *odemensis* (Ladizinsky) Ferguson, Maxted, van Slageren & Robertson

L. ervoides (Brign.) Grande

L. nigricans (M. Bieb.) Godron

L. lamottei Czefr.

Centres of diversity have been identified in four wild *Lens* taxa: *L. orientalis* has two centres of diversity in south-eastern Turkey and north-western Syria, and southern Syria and northern Jordan; Centre of origin of *L. odemensis* overlaps with that of *L. orientalis* in southern Syria and northern Jordan; *L. ervoides* has its centre of genetic diversity in eastern Mediterranean cost and former Yoguslavia; and *L. nigricans* has its centre of diversity in south-west Turkey and along the coast of former Yugoslavia, France, and Spain (Davies et al., 2007).

Based on more recent genotyping-by-sequencing (GBS) and phylogenetic studies, above mentioned *Lens* species have been classified into four distinct gene pools (Wong et al., 2015):
Primary gene pool: *L. culinaris, L. orientalis, L. tomentosus*
Secondary gene pool*: L. lamottei, L. odemensis*
Tertiary gene pool: *L. ervoides*
Quaternary gene pool: *L. nigricans*

Members of the same gene pool are more likely to produce viable seeds when crossed with each other while crossing between members of different gene pools often fails due to embryo abortion (Davies et al., 2007). Members belonging to the primary gene pool are thus the most genetically accessible as their hybridization success with *L. culinaris* is very high. While plant growth regulators and embryo rescue techniques can now be used to successfully intercross most wild *Lens* species in the primary, secondary and tertiary gene pools with *L. culinaris* (Davies et al., 2007), the exception to date is *L. lamottei.*

Wild relatives of cultivated lentil have the potential of introducing immense genetic variation in the otherwise narrow genetic base of *L. culinaris* and will be important in identifying novel genes that can be introgressed into cultivated lentil to address future needs of lentil production (Davies et al., 2007; Redden et al., 2007; Wong et al., 2015).

2.2 Trichomes and their classification

Trichomes are hair-like projections that originate from aerial epidermal cells. Trichomes can be unicellular or multicellular, and they can be found on various surfaces of the plant including leaves, stems, roots, petals, sepals, stamens, gynoecia and fruits; and can exhibit great diversity of origin, size, location, morphology, surface microstructure, secretion capability, function, etc. (Werker, 2000). Trichomes are classified as "glandular" or "non-glandular" on a morphological basis of either presence or absence of a secretory head (Santos Tozin et al., 2016; Werker, 2000). Glandular trichomes of plants are traditionally considered to be involved in production, excretion, storage, or absorbance of substances, while non-glandular trichomes serve as a physical barrier for biotic and abiotic stresses (Santos Tozin et al., 2016; Werker, 2000). However, a recent study has shown that in some species of *Lamiaceae* and *Verbenaceae*, non-glandular trichomes supplement the function of glandular trichomes by producing bioactive compounds such as lipids, polysaccharides, terpenes, alkaloids, and phenolic compounds (Santos Tozin et al., 2016).

Glandular and non-glandular trichomes are further classified according to their morphology and function. They can be uniseriate, biseriate, or multiseriate, and can be variably shaped, for example, stellate, T-shaped, dendritic, hooked, rounded, etc. (Werker, 2000). Figure 2.1 illustrates the types of trichomes found on the aerial surfaces of *Colquhounia* spp., redrawn from Hu et al. (2012). Different types of trichomes can be found on the same plant and even on the same organ, and similar types of trichomes can serve different purposes based on their location and the ontogeny of the plant (Werker, 2000).

Trichome density can also vary on the same plant tissue, depending on its location. For example, a study by Dahlin et al. (1992) of three cultivars of common bean (*Phaseolus vulgaris* L.) revealed the presence of straight, hooked, and glandular trichomes on both abaxial and adaxial leaf surfaces, although the density of each type of trichome differed based on the cultivar and on the surface examined (Dahlin et al., 1992). A similar study conducted by Stenglein et al. on four types of Mesoamerican common bean reported that the abaxial leaf surface always had more trichomes and higher density of larger stomata than the adaxial leaf surface (Stenglein et al., 2004).

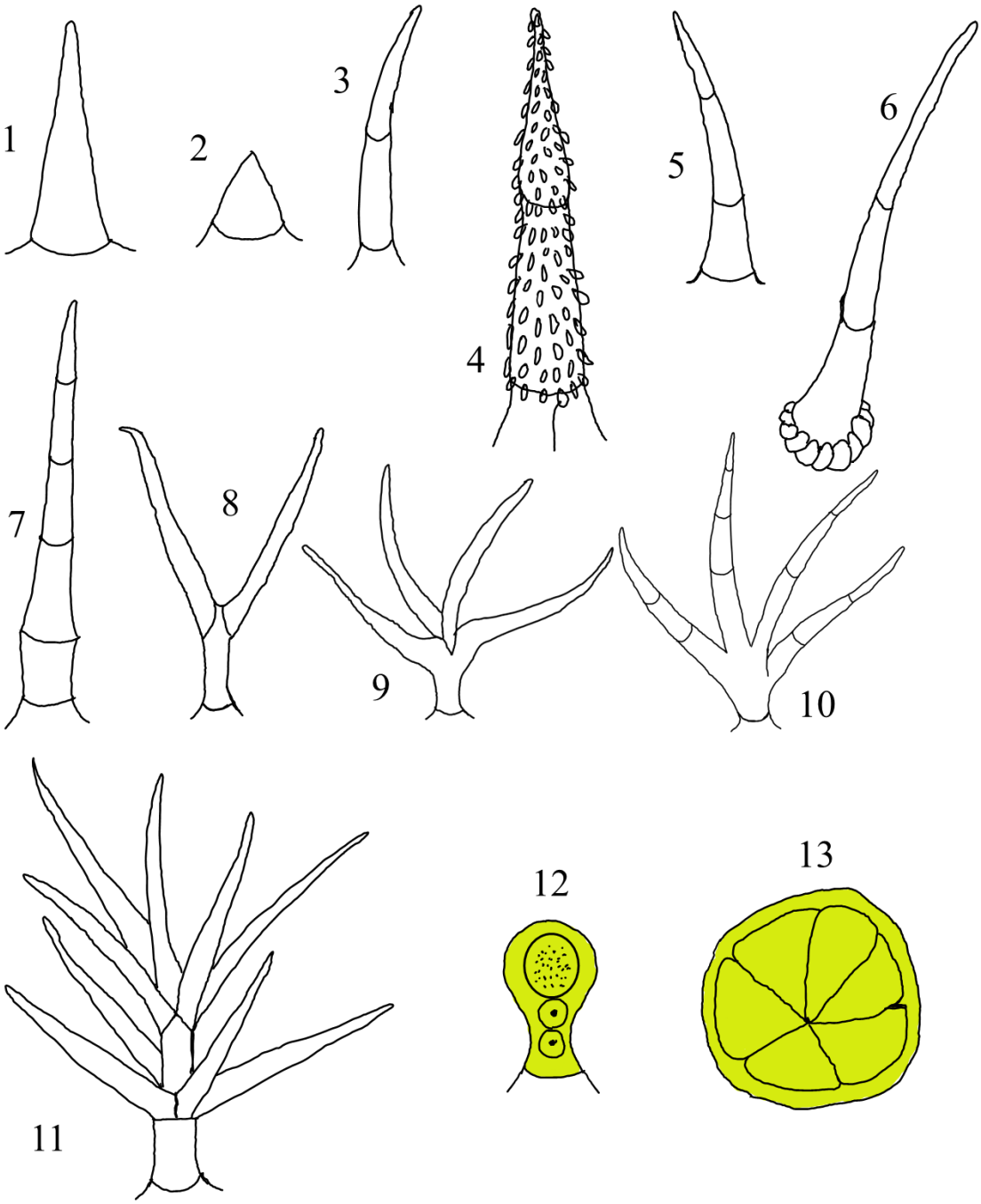


Figure 2.1 Illustration of different types of trichomes found in *Colquhounia* spp. (1, 2) unicellular trichomes (leaves of *C. seguinii*); (3) two-celled trichome (leaves of *C. elegans* var. *elegans*); (4) two-celled trichome with wall protuberances (stems of *C. seguinii*); (5) three-celled trichome (leaves of *C. seguinii* var. *pilosa*); (6) three-celled trichome with 12 to 16 cells surrounding the basal cell (leaves of *C. elegans* var. *elegans*); (7) more than three-celled trichome (leaves of *C. elegans* var. *elegans*); (8) biramous trichome (leaves of *C. coccinea* var. *mollis*); (9) stellate trichome with unicellular branches (leaves of *C. coccinea* var. *mollis*); (10) stellate trichome with multi-cellular branches (leaves of *C. compta* var. *mekongensis*); (11) dendroid trichome (leaves of *C. elegans* var. *elegans*); (l2) apical view of capitate glandular trichome (leaves of *C. elegans* var. *tenuiflora*); (13) peltate glandular trichome (leaves of *C. seguinii* var. *seguinii*). Glandular trichomes are coloured green. Drawings not to scale. Redrawn from Hu *et al.* (2012).

Displaying such a vast diversity in their structure, function, and density, the trichomes on aerial surfaces of plants are an important trait because of their following three functions: defence against abiotic/environmental factors, defence against biotic factors such as insects and pathogens, and metabolic and physiological effects exerted by the compounds released by trichomes (Savé et al., 2000). This review will focus on the defensive role played by trichomes during the abiotic stress of drought, impact of trichomes on herbicide efficacy, and influence of trichomes on the biotic stress of insect herbivory.

2.3 Role of trichomes in drought resistance

Trichomes of plants commonly found in the semi-arid environments play a protective role against drought. Non-glandular trichomes, through forming a dense blanket on the leaf surface, reflect solar radiation and prevent the plant from absorbing heat, which in turn reduces the need for transpirational cooling in plants (Ehleringer and Björkman, 1978; Huttunen et al., 2010). Cacti, which are highly adapted to growing in deserts, absorb water at dawn from the fog through their trichomes and store it in their stems filled with mucilage (Ju et al., 2012). Multicellular trichomes on the upper leaf surface of pineapple also absorb and conduct water (Sakai and Sanford, 1980).

In *Lychnophora diamantinana*, a plant in the daisy family native to a Brazilian region that receives high solar radiation and suffers from water deficits, the glandular trichomes secrete hyaline, a highly viscous material that protects the young organs of the plant from desiccation (Lusa et al., 2014).

In *Olea europea,* a drought tolerant cultivar was found to have increased trichome and stomatal density, along with lower leaf area (Guerfel et al., 2009). Similarly, a Chinese field study of *Caragana korshinskii*, a leguminous plant native to Asia, revealed that plants grown in dry condition had larger, denser trichomes on leaves compared to plants grown in region with high precipitation (Ning et al., 2016). In a study comparing two soybean isolines differing in trichome density, evapotranspiration was reduced in the densely pubescent isoline, resulting in greater water use efficiency (Baldocchi et al., 1983). However, increased trichome density has not always associated with increased drought tolerance. A study of *Arabidopsis lyrata* found no association between drought tolerance and trichome density, leading the authors to speculate that drought tolerance might have been related to leaf wax production, ratio of leaf area to volume, root/shoot ratio, and root and shoot morphology (Huttunen et al., 2010).

2.4 Role of trichomes in insect resistance

Resistance mechanisms employed by host plants against insect herbivores include tolerance, antibiosis, and antixenosis. Antixenosis is defined as behavioural influence through specific traits that make plants unattractive. Antibiosis occurs when growth, survival, and fecundity of the insect are negatively affected by the plant. Tolerance is an inherent trait of the plant that enables it to remain healthy and withstand pest damage by increasing its ability to heal wounds and repair damage – all without exercising selection pressure on the insect or affecting the insect at all (Nalam et al., 2019).

Trichomes comprise the first line of defence for plants, aiding in protecting the plant against insect pests. The layer of both glandular and non-glandular trichomes on the aerial surface of plant tissues serves as a physical barrier against insects by disrupting their movement and limiting their access to the soft epidermal tissue (Agrawal et al., 2009). Non-glandular hooked trichomes efficiently entrap small insects or spear their bodies to prevent them from feeding (Xing et al., 2017). Glandular trichomes secrete biologically active secondary metabolites that include terpenoids, fatty acid derivatives, polyketides, and phenylpropanoids. These exudates, due to their stickiness, toxicity, odor, or taste, are able to repel, trap, or kill insects, thus preventing them from feeding on the plant (Schilmiller et al., 2008; Wheeler and Krimmel, 2015). Adhesive and viscous exudates from trichomes in some plants result in a sticky surface that enhances the number of herbivorous insects sticking to it, which in turn attracts their predators, thereby providing indirect defence to the plant against herbivorous insects (Krimmel and Pearse, 2013).

2.4.1 Trichome-mediated insect resistance in legumes/pulse crops

Both glandular and non-glandular trichomes can influence herbivory directly in legumes. A recent study done in *Phaseolus vulgaris* (common bean) revealed that adult *Liriomyza trifolii* (leaf miners) were entrapped by non-glandular hooked trichomes, and that their mouthparts were most susceptible to the trichomes, followed by legs and ovipositors (Xing et al., 2017). The plants' capture efficiency was highest during the vegetative state followed by the fruiting and cotyledon stages. The lower/ventral leaf surface (with highest density of trichomes) was the most efficient in capturing leaf miners (Xing et al., 2017). Capturing efficiency was dependent on plant developmental stage as well as trichome density. These results concur with previous studies in soybean (*Glycine max*) with *Cerotoma trifurcata* (Förster) (adult leaf bean beetle) (Lam and Pedigo, 2001), potato leafhopper (*Empoasca fabae* (Harris)) (Elden and

Lambert, 1992), cabbage looper (*Trichoplusia ni* (Hübner)) (Khan et al., 1986), and soybean pod borer (*Etiella zinckenella* (Treitschke)) (Permana et al., 2012). These studies show that there is a negative correlation between trichome density and oviposition and/or damage by insect feeding on leaves and pods.

Glandular trichomes are also found in legumes, and their exudates have been found to have a negative effect on insects. *Stylosanthes* species secrete a viscous compound that in vapour form immobilizes larvae of cattle ticks (Sutherst et al., 1982). These compounds were subsequently found to be non-polar and lipophilic, with linoleic acid in highest abundance (Muro Castrejón et al., 2003). Glandular trichomes of *Medicago sativa* were also found to secrete compounds that deter potato leafhopper (Ranger et al., 2004); and in cowpea (*Vigna unguiculata*), a significant negative correlation was found between trichome density and infestation of pods by legume pod borer, *Maruca testulalis* (Oghiakhe et al., 1992).

High trichome density (or pubescence) is not always effective in defending legumes against insects. A study of lentil (*L. culinaris* Medik.), found that high infestation of black aphids (*Aphis craccivora* Koch) occurred on genotypes with highly pubescent leaves, while genotypes with a slight pubescence on their leaves had the lowest aphid infestation (Kumari et al., 2009). They explained these results based on the reasoning that trichomes might enable better attachment of black aphids to leaves and prevent their nymphs from falling off, thus facilitating their stay on the leaves.

Though pubescence has variable effects on the infestation of plants by insect herbivores, it is a trait that can be used in plant breeding programs to influence insect feeding behaviour, and ultimately impart partial or complete resistance to the plant against particular insects (Miklas et al., 2006).

2.4.2 Effect of pubescence on aphids

Aphids (order Hemiptera) are among the most economically important pests of agricultural crops in the world (Sorensen, 2009). They have piercing-sucking mouthparts and are specialized in feeding on the plant phloem, draining the plants of their nutrients and causing them to wither and die (Smith and Chuang, 2014; Sorensen, 2009). Moreover, aphids also serve as vectors for a variety of devastating plant viruses such as the pea enation mosaic virus, potato leaf roll virus, broad bean wilt virus, etc. (Ng and Perry, 2004).

Studies have shown that both glandular and non-glandular trichomes mediate resistance to aphids via antibiosis and/or antixenosis (Smith and Chuang, 2014). Glandular trichomes, through their exudates, have been shown to deter and entrap aphids on alfalfa, potato, tomato, (Avé et al., 1987; Goffreda et al., 1989; Shade and Kitch, 1983); and non-glandular trichomes have been shown to slow down aphid movement and repel aphids in wheat (Roberts and Foster, 1983; Webster et al., 1994).

2.4.3 Pubescence effects on pea aphid

The pea aphid, *Acyrthosiphon pisum*, is specialized to feed on field pea, alfalfa, and clover, but its other hosts include pulse crops such as lentil, faba bean, dry bean, etc. (Gavloski, 2017). Like other aphids, pea aphids feed by penetrating plant tissues with their stylets, after which they probe through the layers of mesophyll and epidermal cells until they reach the phloem sieve element (Gao et al., 2008). Pea aphids are soft bodied, pear shaped, 3 mm long and 1.5 mm wide, and their colour ranges from light to dark green (Manitoba Agriculture, 2017). They are wingless and have a parthenogenetic life cycle in the summer, giving birth to live female nymphs which are smaller but closely resemble adults (Gavloski, 2017). Young nymphs mature in 5-15 days from birth in the summer. The optimum temperature for rapid pea aphid development is 23-28°C, and a female aphid can give birth to up to 12 live nymphs per day (Brisson and Davis, 2008; Brisson and Stern, 2006; Gavloski, 2017). Due to their short generation time, pea aphids inflict substantial yield and economic damage on lentil and other hosts by voiding the plants of nutrients and transmitting pathogenic viruses, and are reported to decrease lentil yield by up to 7% in the USA (Davis et al., 2015; Elbakidze et al., 2011; Gao et al., 2008).

Few studies have investigated the effect of pubescence on pea aphids, and the results have varied depending on the species. In *Medicago sativa*, the glandular trichomes of wild accessions reduce fecundity and growth of pea aphids (Shade and Kitch, 1983). However, Gao et al. (2008) assessed the probing and feeding behaviour of pea aphid on two near-isogenic lines of *M. truncatula*: A pea aphid resistant line called, ‘Jester’, and a pea aphid susceptible line, ‘A17’ (Gao et al., 2008). Through comparing the non-probing behaviour and locomotion of pea aphids on both of these lines, they found no significant difference, and thus concluded that neither trichomes nor other cell wall properties such as epicuticular wax composition or thickness confer resistance to ‘Jester’ against pea aphid (Gao et al., 2008).

2.5 Chemical weed control in lentil

Lentil is non-competitive with weeds due to its short stature and slow growth in the early stages of development, thus lending itself susceptible to severe yield losses due to weeds (Brand et al., 2007; Smitchger et al., 2012). Moreover, there are only a few herbicides registered in Canada for use in lentil to control broadleaf weeds, with the only postemergence options being (1) metribuzin (Photosystem II inhibitor, Group 5) and (2) imidazolinones (ALS enzyme inhibitors, Group 2), which are used on imidazolinone tolerant Clearfield™ lentil varieties (Saskatchewan Ministry of Agriculture, 2020). The 5-10 node stage has been identified as the critical period of weed control (CPWC) in lentil, i.e., if weeds are controlled within this period of lentil development, yield loss can be prevented (Fedoruk et al., 2011). There have been efforts to increase herbicide tolerance in lentil via genetic modification (Gulati et al., 2002; Rizwan et al., 2017). Cultivars with increased herbicide tolerance could lead to an increase in CPWC and allow for a larger window for chemical weed control in lentil.

2.6 Effect of pubescence and addition of adjuvants on herbicide efficacy

Since the first barrier to any substance sprayed on the plant is the cuticle, one way to increase herbicide resistance in plants is by altering cuticle properties so as to reduce penetration of the herbicide after application (Délye et al., 2013). Based on density and type, trichomes protruding from the epidermis might also serve as an additional layer of barrier against foreign substances by influencing their deposition, distribution, and retention before they reach the leaf surface (Hess and Falk, 1990; McWhorter et al., 1995). Large herbicide droplets were observed to shatter upon impact with the stellate hairs of turkey mullein (*Eremocarpus setigerus* Benth.) and small herbicide droplets lodged on the trichome surface, resulting in only a few droplets reaching the leaf surface (Hess et al., 1974). A significant positive correlation was also found between trichome density on the adaxial surface and herbicide (glyphosate) resistance in wild India mustard (*Brassica juncea*) population (Huangfu et al., 2009). In the case of the common perennial weed silverleaf nightshade (*Solanum elaeagnifolium*), its dense covering of stellate hairs repelled water-based tracer dyes and did not facilitate transport of tracer dyes into the vascular tissues, although transport was facilitated by the addition of adjuvant (Burrows et al., 2013). In studying trichomes and their interaction with water droplets in subalpine and montane plants, Brewer and Smith (1997) observed three basic types of interactions, illustrated in Figure 2.2: (1) Low trichome density (< 1 trichome/mm^2) was associated with no effect on droplet formation and retention, and a film of moisture was observed on the epidermis (Figure 2.2 a); (2) Medium-low trichome density (5-20 trichomes/mm^2) was associated with ‘segregating

strategy' where water remained in pools at the base of hydrophilic trichomes awaiting removal via evaporation (Figure 2.2 b); and (3) High trichome density (> 20 trichomes/mm^2) was associated with 'lifting strategy' where water droplets were suspended above the dense trichome layer and was prevented from reaching the leaf epidermis (Figure 2.2 c) (Brewer and Smith, 1997).

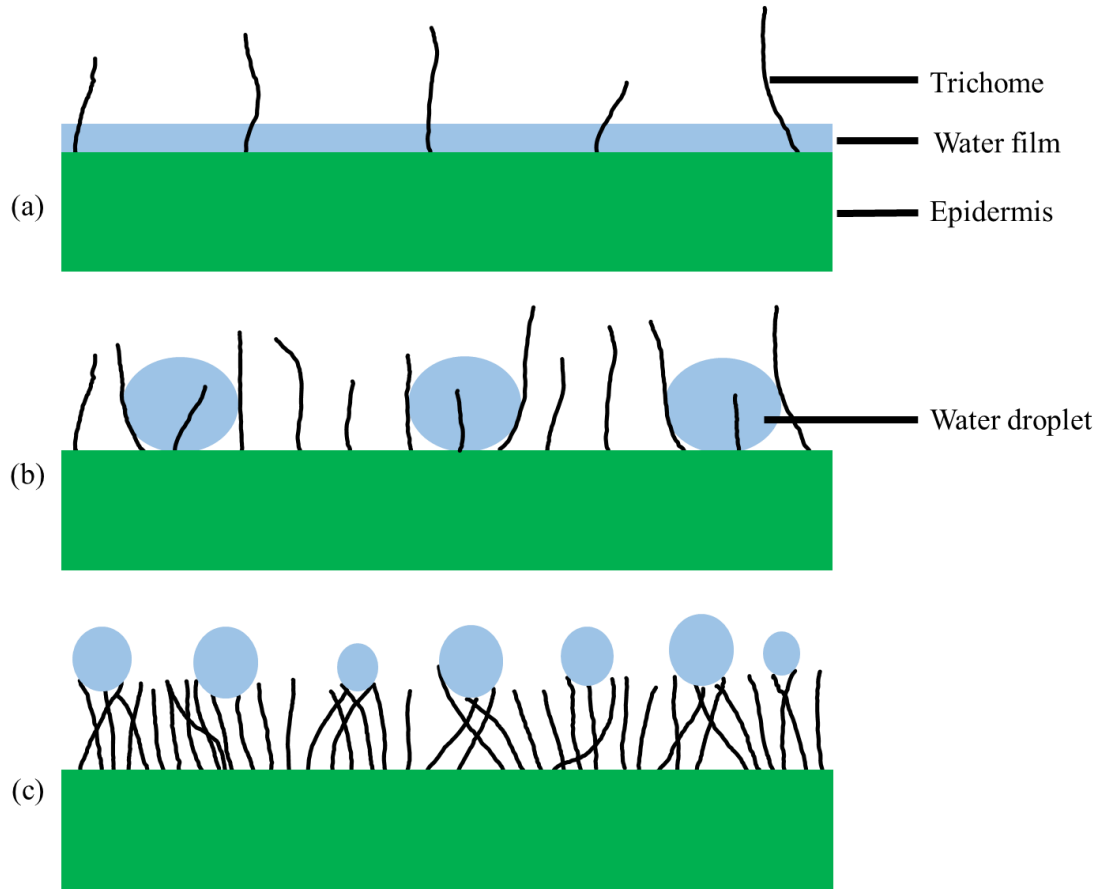


Figure 2.2 Three types of water-trichome interactions. (a) No interaction between trichomes and water. (b) Water droplets accumulate in patches encircled by trichomes. (c) Water droplets remain suspended above the trichomes. Redrawn from Brewer and Smith (1997).

Adjuvants in the form of surfactants are often added in foliar applications to increase wetting ability of the spray, thereby reducing surface tension and contact angle of the spray droplets, resulting in improved efficacy (Gitsopoulos et al., 2014; Singh et al., 1984; Singh and Singh, 2005; Zhu and Lin, 2016). Surfactants also increase solubility of herbicides in water (Temple and Hilton, 1963) and reduce the droplet evaporation time enabling greater retention and adsorption of the spray droplet on the leaf surface (Yu et al., 2009). In oat, a study showed that surfactants increased herbicide efficacy by increasing absorption independent of spray retention (Ramsdale and Messersmith, 2002). Adjuvants in the form of surfactant thus have the potential to increase herbicide efficacy both in the presence as well as absence of pubescence.

Chapter 3

Characterization of trichomes and other surface microstructures in *Lens* spp. and their influence on water relations under moderate drought

3.1 Introduction

Lentil is an environmentally and economically valuable crop in the Canadian prairies. However, sustained lentil production in the Canadian prairies is threatened by projection of frequent drought in the latter half of the 21st century due to climate change (Yusa et al., 2015). Since wild relatives of cultivated lentil are mostly native to and thrive in the Mediterranean climate of Turkey, Spain, and Syria, scientists are looking to access genetic diversity of wild lentil species to make cultivated lentil withstand drought stress (Gorim and Vandenberg, 2017).

One of the major ways in which water stress affects plant growth and development is by altering water relations within the plant, leading to water loss and subsequent cell collapse due to loss in turgor pressure (Sánchez-Rodríguez et al., 2010). Since most water loss in plants takes place via evaporation through stomatal pores during transpiration (Aston and Jones, 1976), plants that inhabit arid regions have morphological traits that prevent surface water loss and minimize transpiration, enabling them to adapt to drought (Shields, 1950). Some of these anatomical alterations are inducible such as increased trichomes and a waxy cuticle which serve as physical barriers and prevent water loss due to transpiration as well as reflect solar radiation thus keeping the plant cool (Huttunen et al., 2010; Seo and Park, 2011); as well as lower surface to volume ratio of leaves brought about by reduced cell size, thicker cell walls, and increased frequency of stomata (Shields, 1950).

This study aimed to first determine if wild and cultivated lentil genotypes alter their transpiration rate when grown under fully watered (FW) and 40% field capacity (40% FC, or moderate drought) condition. The second objective of this study was to characterize surface microstructures (trichomes, epidermal cells, and stomata) on adaxial surface of wild and cultivated lentil leaflets and determine how their densities are affected when lentil species are grown under moderate drought, and if they explain altered transpiration rate.

3.2 Materials and methods

3.2.1 Plant material and experimental design

The experiment was conducted under controlled conditions in the Phytotron facility at the University of Saskatchewan, Saskatoon, Canada (52°07'58.8"N, 106°37'51.6"W). Table 3.1 provides details of the wild and cultivated genotypes used for this experiment, including their most recent gene pool classification based on Wong et al., 2015. Seeds of wild and cultivated species were scarified, washed in bleach and Tween, and germinated in 50 ml Erlenmeyer flasks in the dark at 22°C. Once germinated, seedlings were planted in 2-gallon plastic pots filled with Sunshine Mix 4 (Sun Gro Horticulture, Canada). Prior to filling pots, the moisture content of each bag of growth medium was determined using a Sartorius MA30 moisture analyzer (Sartorius AG, NY, USA), and the amount placed in each pot was adjusted so that each pot contained 525 g of dry growth medium. Filled pots were kept in a Conviron GR48 growth chamber (Conviron, Winnipeg, Canada) set at 16 h day at 21°C, 8 h night at 15°C, at ambient humidity. 100% field capacity (FC) was determined by randomly choosing four pots that were then filled with water to saturation. Their tops were covered with aluminum foil and the pots were left in the growth chamber for 24 h until no water was draining from the bottom. Each pot was then weighed, and the weight of the empty pots and growth medium was subtracted from their weight to determine the amount of water contained in fully wet pots, hereby referred to as amount of water at 100% FC. Based on these measurements, the amount of water reduction required to reach 80% FC and 40% FC was calculated. To avoid flooding of the pots, 80% FC was considered as the fully watered condition for the experiment, and 40% FC was considered the moderate drought condition. 500 mL of Hoagland solution was added to each pot before planting. The Hoagland solution contained 5 mM KNO_3, 5 mM $Ca(NO_3)_2$, 2 mM $MgSO_4.7H_2O$, 2 mM KH_2PO_4, 45 µM Fe chelate (containing $FeSO_4.7H_2O$ and EDTA $2Na.2H_2O$), and micronutrients (9.1 µM $MnCl_2.4H_2O$, 46.3 µM H_3BO_3, 0.76 µM $ZnSO_4.7H_2O$, 0.32 µM $CuSO_4.5H_2O$, and 0.1 µM $Na_2MoO_4.2H_2O$).

Table 3.1 Species, genotype, centre of origin and ecological conditions at the centre of origin of 20 lentil accessions used in the experiment.

Species	Genotype	Centre of origin[a]	Ecological conditions at centre of origin[a,c]	Gene pool[b]
L. culinaris	Eston	Cultivated lentil from Turkey	Mediterranean climate with dry sub-humid conditions in most of Turkey and semi-humid and humid climates along the coastal regions. Annual mean temperature ranges from 4°C to 20°C. Annual average rainfall is around 648 mm occurring mostly during winters (Deniz et al., 2011).	*Primary gene pool*
L. culinaris	CDC Redberry	Cultivated lentil developed by Crop Development Centre, Saskatchewan, Canada	Semi-arid climate with short, warm summers and occasional rains and thunderstorms, and long, extreme winters with sub zero temperatures unsuitable for lentil cultivation (Mwakutuya, 2006). Altitude ranges from 436-1067 m (Hopkins, 1938) and annual precipitation ranges from 300-430 mm (Mwakutuya, 2006).	*Primary gene pool*
L. culinaris	Indianhead	Cultivated lentil developed at Crop Development Centre, Saskatchewan, Canada, by selecting seeds of PI 320952 originating from Czechia (Carlisle, 2016)	Generally temperate climate with snowfall at higher altitudes and with decreasing temperatures. Altitude ranges from about 100 to 1600 m above sea level and annual precipitation ranges from 400 to 1400 mm, with most areas receiving 600-800 mm/year (Beranová and Kyselý, 2018; Dubrovsky et al., 2009; Jenicek and Ledvinka, 2020).	*Primary gene pool*
L. culinaris	CDC Greenstar	Cultivated lentil developed by Crop Development Centre, Saskatchewan, Canada	Same as CDC Redberry.	*Primary gene pool*
L. tomentosus	IG 72613	Latitude: 37.9167, Longitude: 40.2333 (Diyarbakir, Turkey)	Hot, dry summers and mild winters. Mean monthly temperature ranges from 2 to 30°C. Altitude of 677 m above sea level and annual precipitation of 488 mm (Aydin et al., 2010; Sessiz et al., 2010; Yürekli, 2015).	*Primary gene pool*

Species	Genotype	Centre of origin[a]	Ecological conditions at centre of origin[a,c]	Gene pool[b]
L. tomentosus	IG 72614	Latitude: 37.9167, Longitude: 40.25 (Diyarbakir, Turkey)	Same as *L. tom.* IG 72613.	*Primary gene pool*
L. tomentosus	IG 72805	Latitude: 37.75, Longitude: 39.7667 (Sanliurfa, Turkey)	Arid region with dust winds and high solar radiation. Average winter and summer temperature of 5°C and 40°C, respectively. Altitude of 1150 m above sea level and annual precipitation of 1514 mm (Elmas, 1994).	*Primary gene pool*
L. orientalis	IG 72643	Latitude: 36.3375, Longitude: 36.8389 (Aleppo, Syria)	Semi-arid climate with hot summers and freezing winters. Average minimum and maximum temperature of 2°C and 35°C, respectively. Altitude of 410 m above sea level (Kattan, 1997) and annual precipitation of 330 mm (Faour et al., 2010).	*Primary gene pool*
L. orientalis	IG 72529	Latitude: 38.6833, Longitude: 39.2333 (Elazig, Turkey)	Arid summers and most precipitation during spring, autumn, and winter seasons. Mean winter and summer temperature is <0°C and 27°C, respectively (van Zeist et al., 1970). Altitude of 1015 m above sea level and annual precipitation of 410 mm (Yürekli, 2015).	*Primary gene pool*
L. orientalis	PI 572376	Latitude: 37.67, Longitude: 29.13 (Denizli, Turkey)	Mediterranean cold semi-arid climate. Average winter and summer temperatures of 1°C and 22-28°C, respectively. Altitude of 1300 m above sea level and annual precipitation of 556 mm (Güvensen et al., 2013).	*Primary gene pool*
L. orientalis	IG 72611	Latitude: 38.6667, Longitude: 42.1667 (Bitlis, Turkey)	Climate classified to be on the boundary of Mediterranean and Humid Continental with excessive snowfall (Aydin and Işhik, 2015). Average monthly temperature in winter and summer is -3°C and 23°C, respectively (Suzuki, 2013); Altitude of 1545 m above sea level and annual precipitation of 1234 mm (Yürekli, 2015).	*Primary gene pool*
L. orientalis	IG 72622	Latitude: 40.5167, Longitude: 34.95 (Corum, Turkey)	Hot, dry summers and cold, rainy winters with mean minimum temperature of -5°C and mean maximum temperature of 28°C (Ertabaklar et al., 2005; Karasartova et al., 2018). Altitude of	*Primary gene pool*

Species	Genotype	Centre of origin[a]	Ecological conditions at centre of origin[a,c]	Gene pool[b]
L. orientalis	IG 72622		801 m above sea level and annual precipitation of 429 mm (Karasartova et al., 2018).	
L. lamottei	IG 110813	Latitude: 37.4167, Longitude: -4.25 (Lucena, Córdoba, Andalucía, Spain)	Mediterranean climate with warm, dry summers and cool, wet winters with average minimum and maximum temperature of 7°C and 30°C, respectively (Galán et al., 1995). Altitude of 660 m above sea level and annual precipitation of 788 mm.	*Secondary gene pool*
L. lamottei	IG 110810	Latitude: 36.75, Longitude: -5.41667 (El Bosque, Cádiz, Andalucía, Spain)	Mediterranean climate with a longer warm, dry season than the cool and wet season (Gracia et al., 2006). Mean minimum and maximum temperature of 6°C and 20°C, respectively. Altitude of 800 m above sea level and annual precipitation of 722 mm.	*Secondary gene pool*
L. odemensis	IG 72623	Latitude: 37.44, Longitude: 41.0167 (Mardin, Turkey)	Mediterranean climate of hot-dry climate zone with average winter and summer temperature of 3°C and 30°C, respectively (Suzuki, 2013; Yilmaz, 2007). Altitude of 1150 m above sea level and annual precipitation of 678 mm (Yürekli, 2015)	*Secondary gene pool*
L. odemensis	IG 72760	Latitude: 32.7092, Longitude: 36.5978 (Sweida, Syria)	Semi-arid climate with hot, dry summers and cold, rainy winters. Altitude of 1010 m above sea level and annual precipitation of about 338 mm (Al Charideh and Abou Zakhem, 2010; Faour et al., 2010).	*Secondary gene pool*
L. ervoides	L-01-827A	Selection from ICARDA (see Fiala et al., 2009)	Assumed same as *L. erv.* IG 72815 (Gorim and Vandenberg, 2017).	*Tertiary gene pool*
L. ervoides	IG 72815	Latitude: 37.6, Longitude: 36.5 (Kahramanmaras, Turkey)	Hot, dry summers and cold and damp winters with average minimum and maximum temperature of 5°C and 28°C, respectively (Doygun, 2009). Altitude of 860 m above sea level and annual precipitation of about 796 mm.	*Tertiary gene pool*
L. nigricans	IG 116024	Latitude: 37.7667, Longitude: 29.1167 (Denizli, Turkey)	Hot, dry summers and cool, rainy winters (Duran and Akyildiz, 2011). Minimum and maximum monthly temperature of 7°C and 25°C, respectively. Altitude of 560 m above sea level and annual precipitation of 340 mm.	*Quaternary gene pool*

Species	Genotype	Centre of origin[a]	Ecological conditions at centre of origin[a,c]	Gene pool[b]
L. nigricans	IG 136640	Latitude: 45.1333, Longitude: 7.05 (Susa, Piemonte, Italy)	Temperate sub-continental climate with warm, humid summers and cold, damp winters (Mutani and Marchetti, 2015). Average minimum and maximum temperature of -1°C and 15°C, respectively. Altitude of about 500 m above sea level and annual precipitation of 1047 mm.	*Quaternary gene pool*

[a]Plant genetic resources accession level data provided by: International Center for Agricultural Research in the Dry Areas (ICARDA), http://www.icarda.org/, Lebanon and Morocco, and USDA National Plant Germplasm System, https://npgsweb.ars-grin.gov, USA. All intellectual property rights (including copyright) in the data are owned and retained by the said institutions. Data accessed through GENESYS Global Portal on Plant Genetic Resources, http://www.genesys-pgr.org, 2020-18-06.

[b]Gene pool classification is based on Wong et al. (2015).

[c]Information for altitude, when not available from other sources, was obtained from Google Maps (http://www.google.com/maps) using the 'Terrain' feature.

The experiment was set up as a randomized complete block design with a total of 328 pots: 20 genotypes with eight replicates per genotype, and two treatments: fully watered (FW) and 40% FC, and eight evaporation pots with growth medium only – four each for FW and 40% FC conditions. Pots were not re-randomized during the experiment. The chamber was set to 16 h day at 21°C, 8 h night at 15°C, at ambient humidity. Light intensity ranged from 276 to 441 µmol m^{-2} s^{-1} depending on the height of the canopy and placement of the pot. Starting in the third week after seedling establishment, all pots were weighed approximately every seven days to determine water loss due to evaporation and transpiration and if needed, water was added to maintain them at their respective intended field capacity.

3.2.2 Collection of surface imprints

During early pod development stage, imprints of the adaxial surface of youngest fully expanded leaflets were collected on Suzuki's Universal Micro-Printing (SUMP) discs (Sump Laboratory, Tokyo, Japan), and were stuck on glass slides using double-sided tape for visualization. The impressions of leaflet surfaces were visualized an EVOS FL inverted microscope (Mill Creek, Washington, United States). Three separate leaflet samples were imaged for each genotype-treatment combination and three fields of view were captured for each leaflet sample. The following data were obtained based on the scale of each image using the software ImageJ (imagej.nih.gov/ij/): trichome density, trichome length, stomatal density, and epidermal cell density. Subsequently, trichome density per unit area and per 100 epidermal cells, and stomatal indices were calculated for each genotype.

The following formula was used to calculate stomatal index:

$$\text{Stomatal index (\%)} = \frac{Number\ of\ stomata\ per\ unit\ area}{Number\ of\ stomata + Number\ of\ epidermal\ cells\ per\ unit\ area} \times 100$$

3.2.3 Determination of water loss due to transpiration

Three plants growing in 40% FC and FW conditions of each of the following 12 genotypes were selected randomly: *L. cul.* Indianhead, *L. cul.* CDC Redberry, *L. cul.* CDC Greenstar, *L. tom.* IG 72613, *L. tom.* IG 72614, *L. tom.* IG 72805, *L. ori.* IG 72643, *L. ori*. PI 572376, *L. lam.* IG 110813, *L. ode.* IG 72623, *L. erv.* L-01-827A, and *L. nig.* IG 116024. From each plant, a stem was cut just above the 5^{th} node (counting from the tip of the stem). The bottom of the cut stem was sealed with vacuum grease to prevent water loss due to evaporation through the exposed incision. The cut stem was held in place upright with a sponge, placed in a 50 mL beaker and put on an analytical scale (Figure 3.2.1). The loss in weight per minute was recorded for the next 20 min as the stem lost water due to transpiration.

Figure 3.2.1 Experimental setup for determining water loss due to transpiration

3.2.4 Scanning electron microscopy

Youngest, fully expanded leaflets were removed from the same 12 genotypes used for transpiration weight loss assay and fixed in 100% ethanol at 4℃ for at least 24 h. The samples were then subjected to critical point drying using CO_2 at approximately 31.5℃ and 1200 psi (lb/in^2) in Polaron E3000 Critical Point Dryer (Quorum Technologies Ltd, East Sussex, United Kingdom), then gold-coated in Edwards S150B Sputter Coater (BOC Edwards, UK) and examined using scanning electron microscopy via Phenom G2 pure (Phenom-World, Eindhoven, Netherlands).

3.2.5 Data analysis

Data were fit using linear mixed model with the interaction of treatment and genotypes as fixed variable and blocking as the random variable. All analyses were done using R Statistical Software (R Core Team, 2019) and significant differences in response variables were calculated using least square means method for multiple comparisons using alpha = 0.05 and adjusting p-value using Tukey method. The function lme from the package lme4 was used to fit the model and the function emmeans from the package emmeans was used to calculate least square means (Bates et al., 2015; Lenth, 2019). Data were plotted using SigmaPlot version 11.0 (Systat Software, San Jose, CA).

3.3 Results

Figure 3.1 shows average weight loss due to transpiration for the 12 genotypes of *Lens* spp. grown under FW and 40% FC conditions, after adjusting for initial shoot weight.

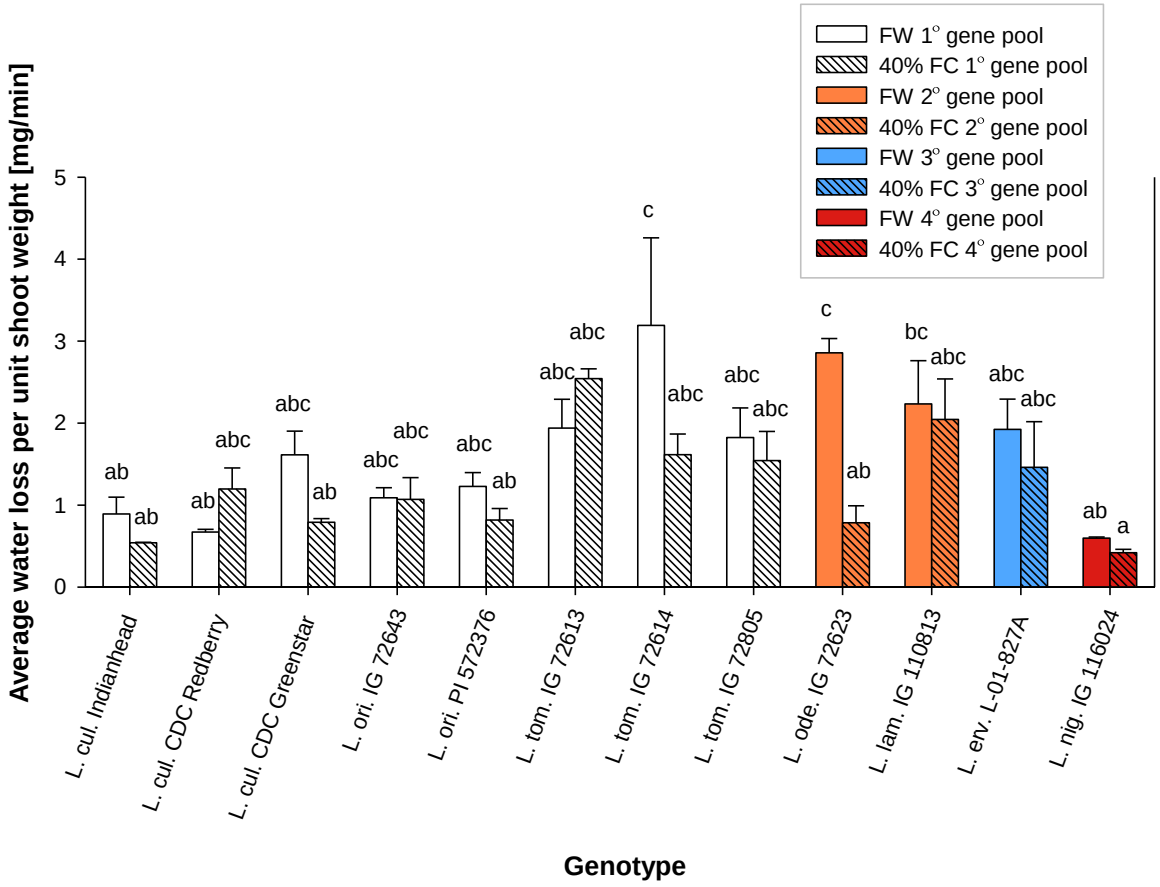


Figure 3.1 Average weight loss due to transpiration in 12 genotypes of *Lens* spp. grown under fully watered (FW) and 40% field capacity (40% FC) conditions. Values are adjusted for initial shoot weight, error bars represent standard errors of treatment means, and compact letter displays show significant differences between means after performing least square means for multiple comparisons using alpha = 0.05 and adjusting p-value using Tukey method.

Performing analysis of variance (ANOVA) on the fitted model indicated that the effect of genotype, treatment, as well as their interaction was significant with $p < 0.05$ (ANOVA table can be found in Appendix 6). For most genotypes, weight loss due to transpiration was higher in plants grown under fully watered condition compared to plants grown under 40% field capacity condition. However, these changes were not significant in any genotype except *L. ode.* IG 72623. Figures 3.2 to 3.13 show weight loss every minute for each of the 12 genotypes. These values have not been adjusted for initial shoot weight at the beginning of the assay. Corresponding scanning electron microscopy images of adaxial leaf surfaces at approx. 180x

are also shown. These images show trichomes on *Lens* spp. being simple, unicellular, hair-like structures without a secretory/glandular head.

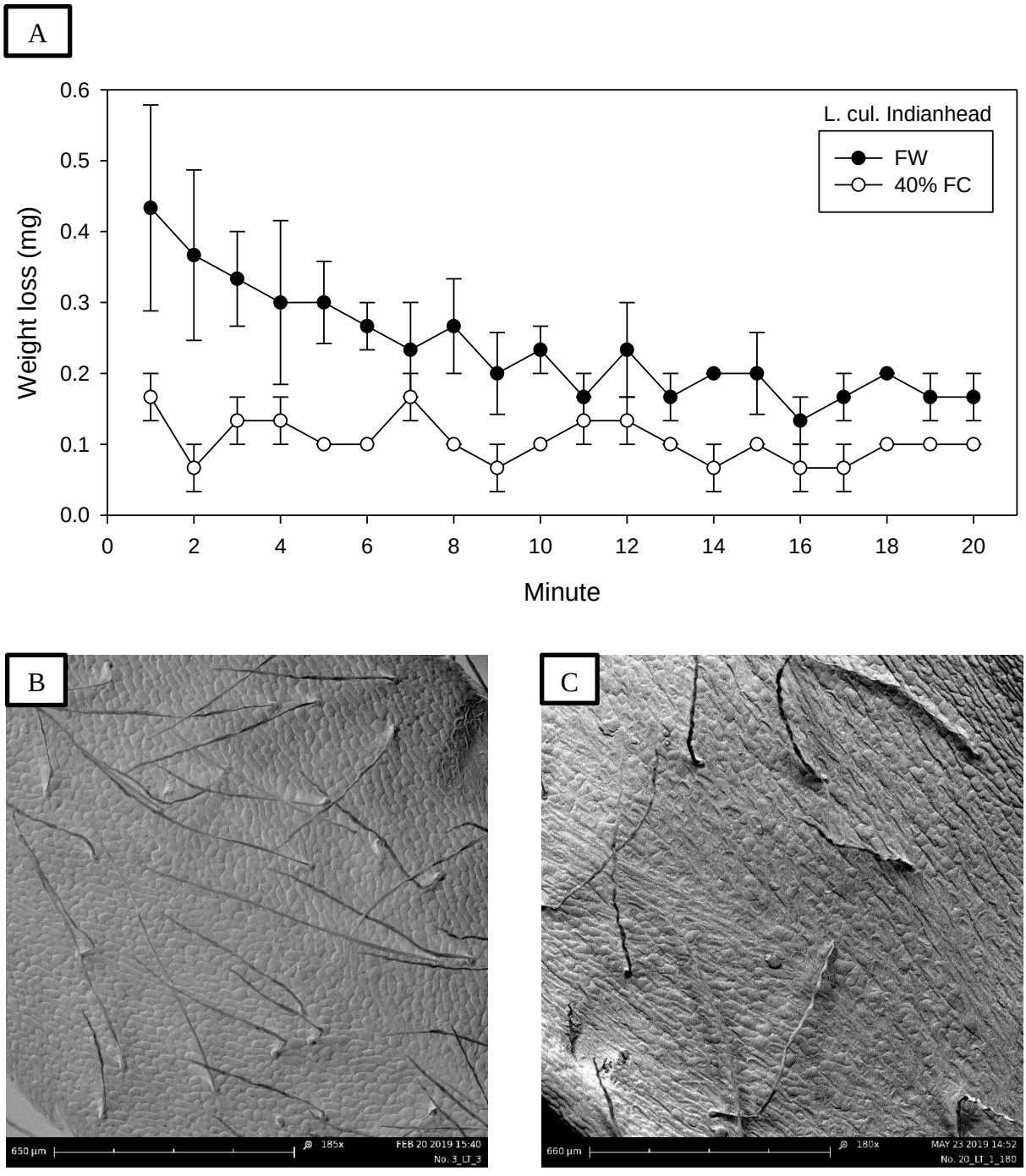


Figure 3.2 (A) Weight loss due to transpiration in *L. cul.* Indianhead grown under fully watered (FW) and 40% field capacity (40% FC) conditions without adjusting for initial shoot weight and error bars representing standard error of means; and scanning electron microscopy images of adaxial leaf surfaces of *L. cul*. Indianhead in (B) FW condition at 185x and (C) 40% FC condition at 180x.

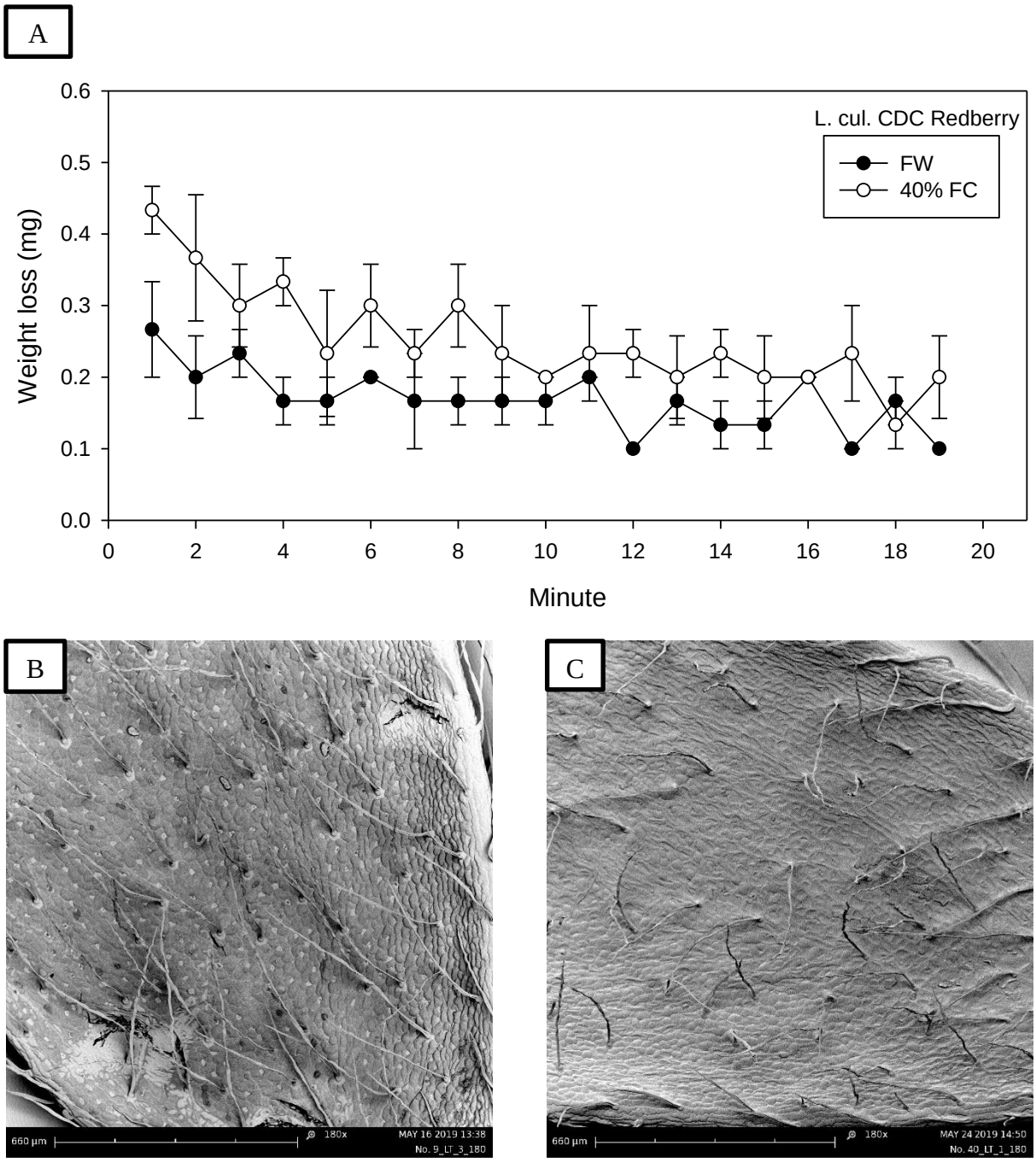


Figure 3.3 (A) Weight loss due to transpiration in *L. cul.* CDC Redberry grown under fully watered (FW) and 40% field capacity (40% FC) conditions without adjusting for initial shoot weight and error bars representing standard error of means; and scanning electron microscopy images of adaxial leaf surfaces of *L. cul*. CDC Redberry in (B) FW and (C) 40% FC condition at 180x.

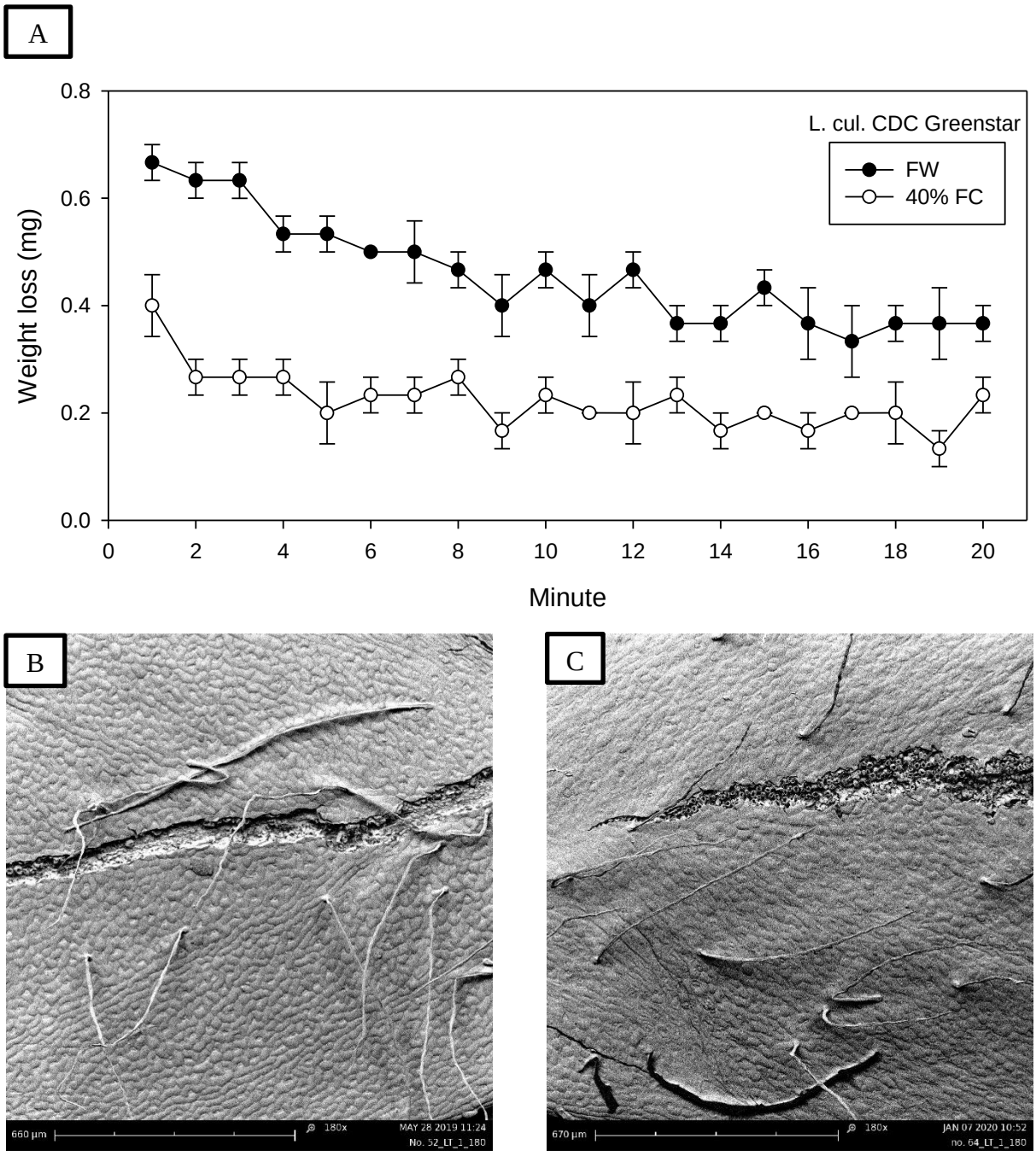


Figure 3.4 (A) Weight loss due to transpiration in *L. cul.* CDC Greenstar grown under fully watered (FW) and 40% field capacity (40% FC) conditions without adjusting for initial shoot weight and error bars representing standard error of means; and scanning electron microscopy images of adaxial leaf surfaces of *L. cul*. CDC Greenstar in (B) FW and (C) 40% FC condition at 180x.

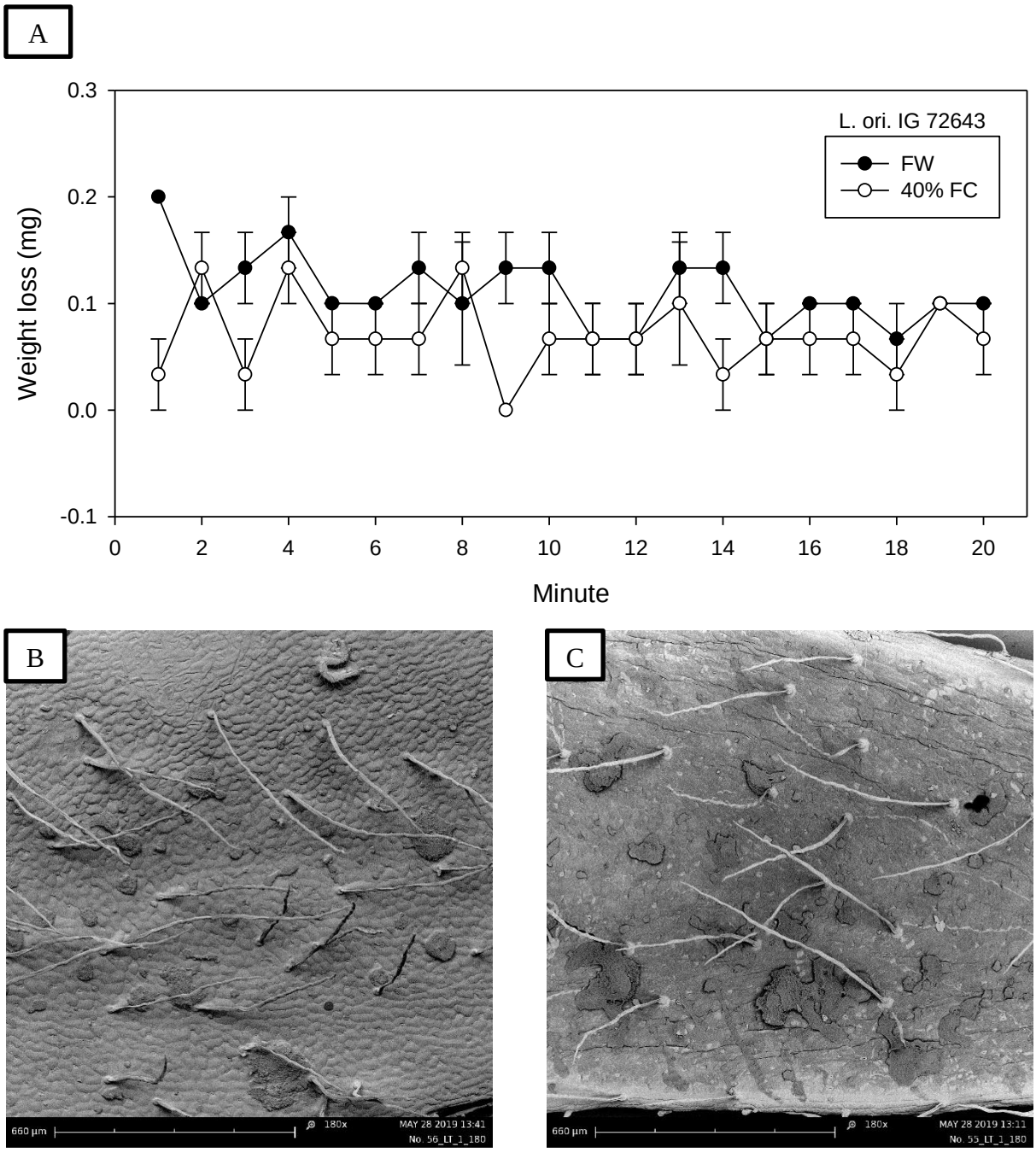


Figure 3.5 (A) Weight loss due to transpiration in *L. ori.* IG 72643 grown under fully watered (FW) and 40% field capacity (40% FC) conditions without adjusting for initial shoot weight and error bars representing standard error of means; and scanning electron microscopy images of adaxial leaf surfaces of *L. ori.* IG 72643 in (B) FW and (C) 40% FC condition at 180x.

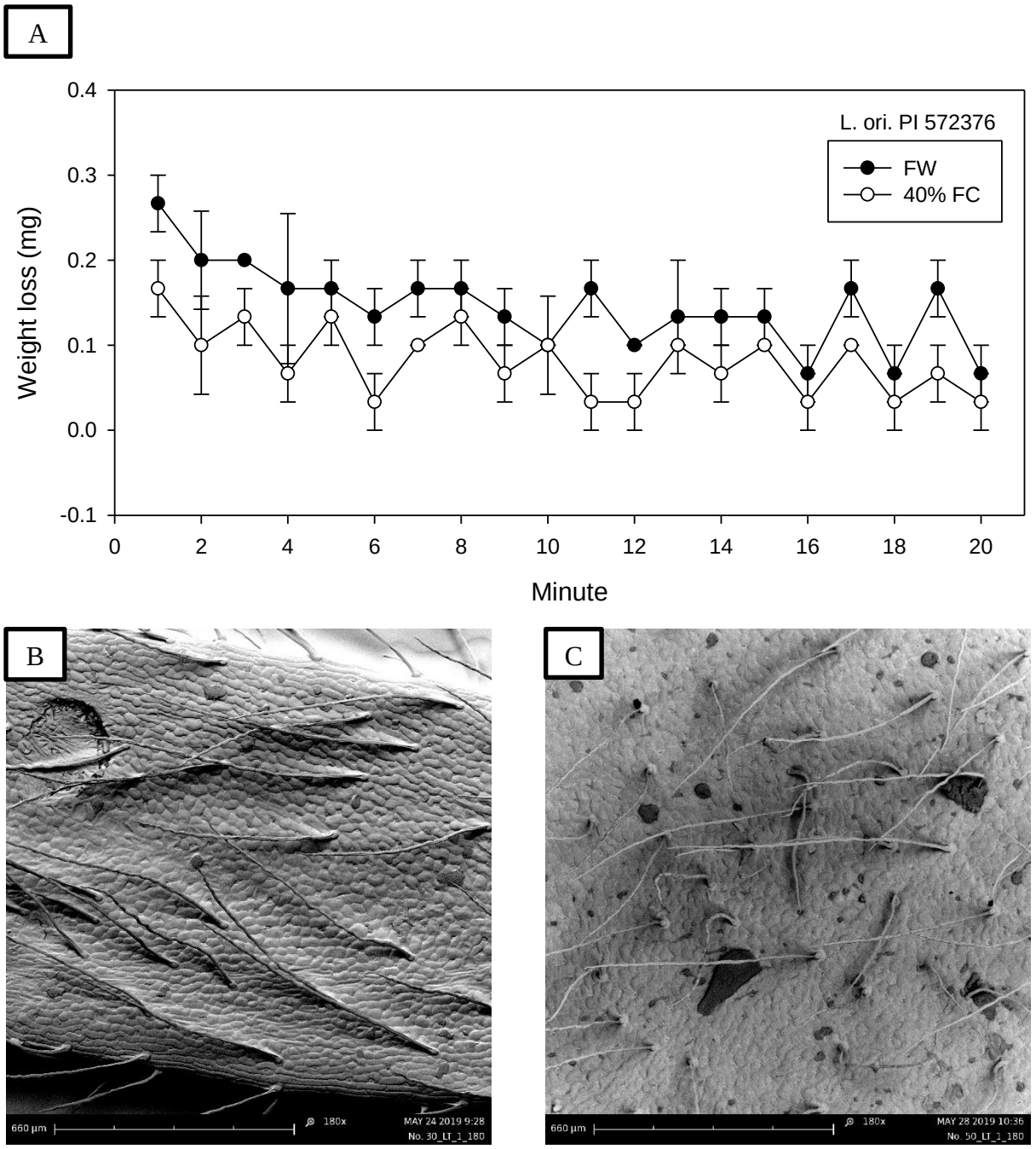


Figure 3.6 (A) Weight loss due to transpiration in *L. ori.* PI 572376 grown under fully watered (FW) and 40% field capacity (40% FC) conditions without adjusting for initial shoot weight and error bars representing standard error of means; and scanning electron microscopy images of adaxial leaf surfaces of *L. ori.* PI 572376 in (B) FW and (C) 40% FC condition at 180x.

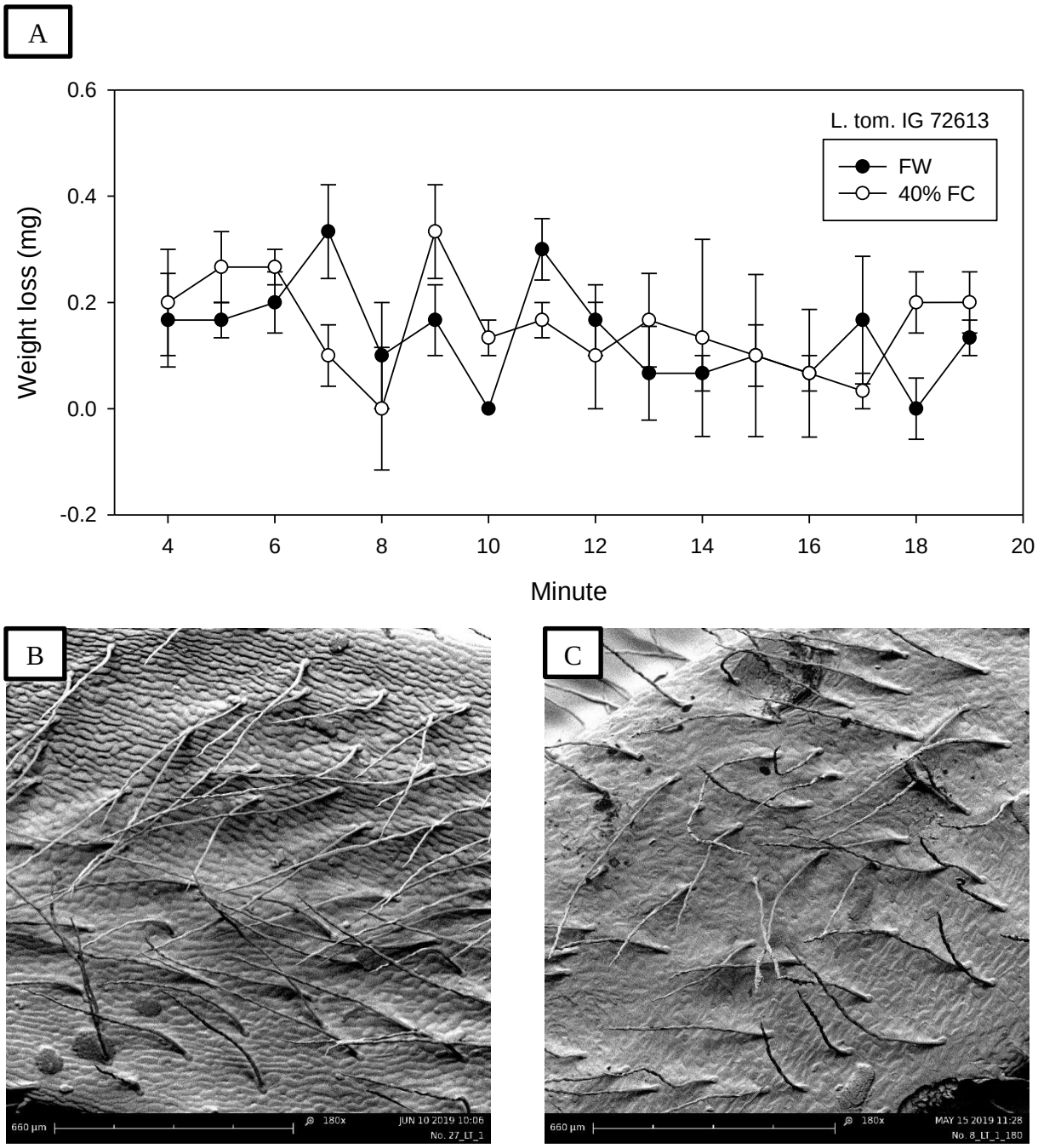


Figure 3.7 (A) Weight loss due to transpiration in *L. tom.* IG 72613 grown under fully watered (FW) and 40% field capacity (40% FC) conditions without adjusting for initial shoot weight and error bars representing standard error of means; and scanning electron microscopy images of adaxial leaf surfaces of *L. tom.* IG 72613 in (B) FW and (C) 40% FC condition at 180x.

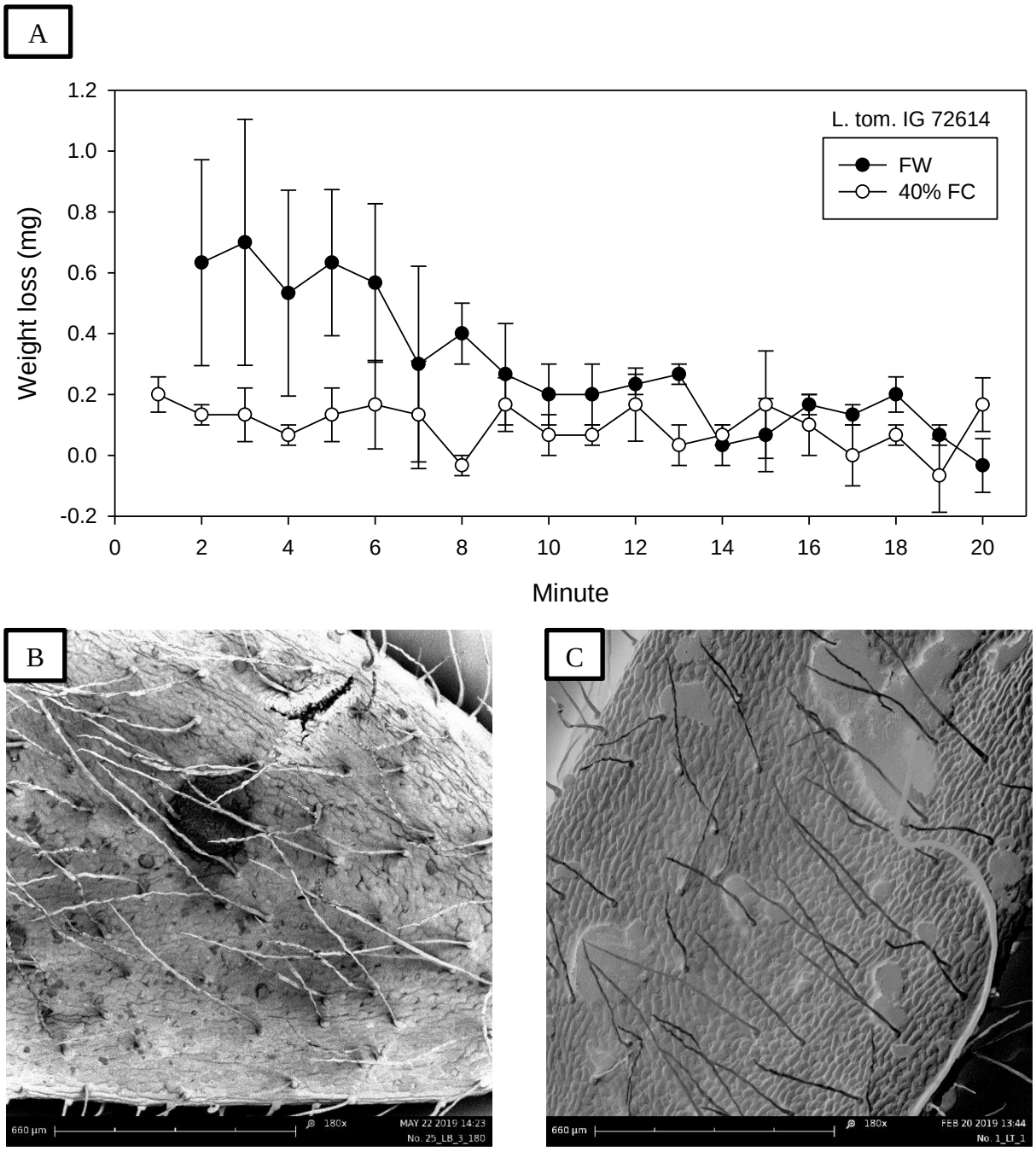


Figure 3.8 (A) Weight loss due to transpiration in *L. tom.* IG 72614 grown under fully watered (FW) and 40% field capacity (40% FC) conditions without adjusting for initial shoot weight and error bars representing standard error of means; and scanning electron microscopy images of adaxial leaf surfaces of *L. tom.* IG 72614 in (B) FW and (C) 40% FC condition at 180x.

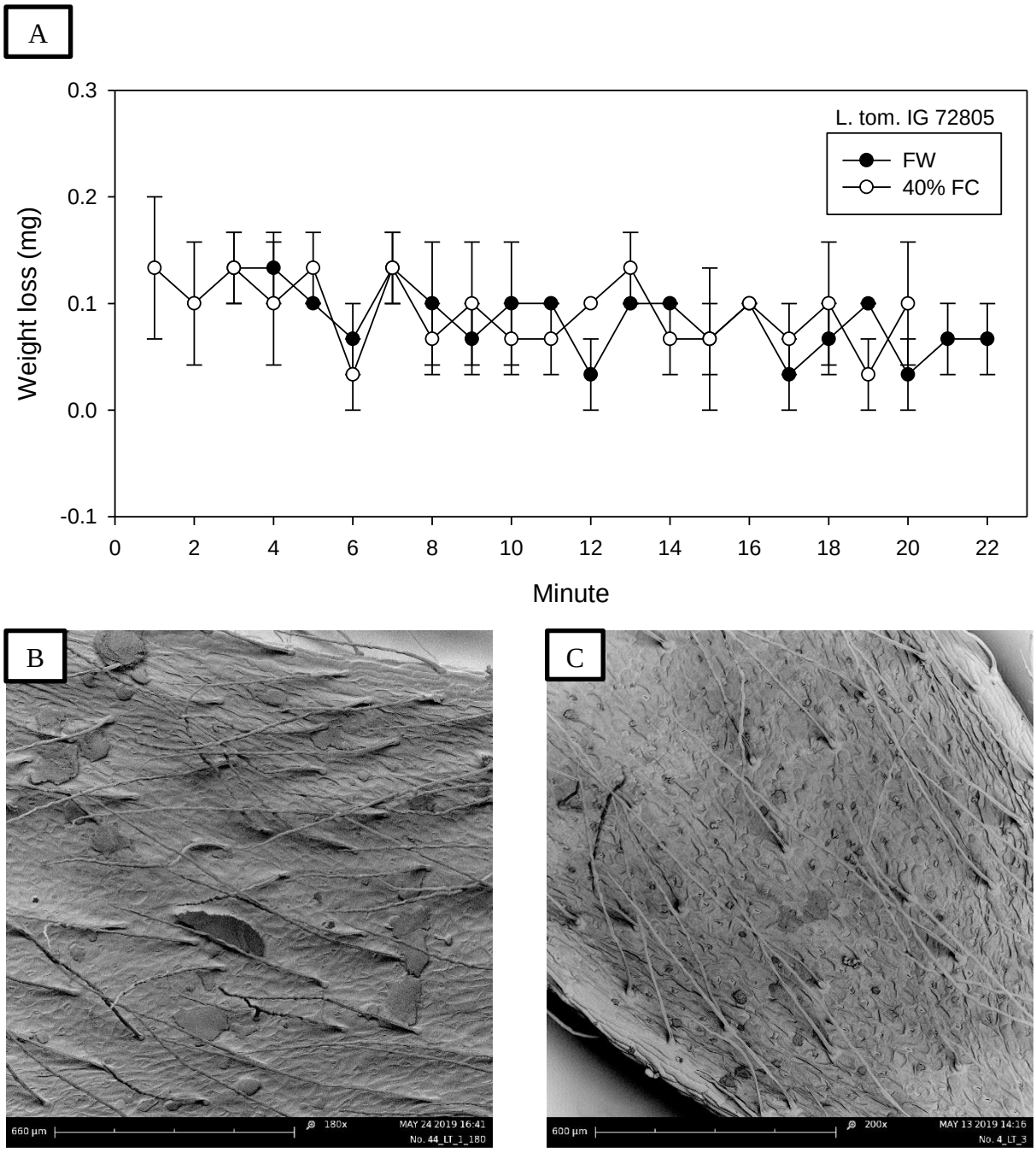


Figure 3.9 (A) Weight loss due to transpiration in *L. tom.* IG 72805 grown under fully watered (FW) and 40% field capacity (40% FC) conditions without adjusting for initial shoot weight and error bars representing standard error of means; and scanning electron microscopy images of adaxial leaf surfaces of *L. tom.* IG 72805 in (B) FW condition at 180x and (C) 40% FC condition at 200x.

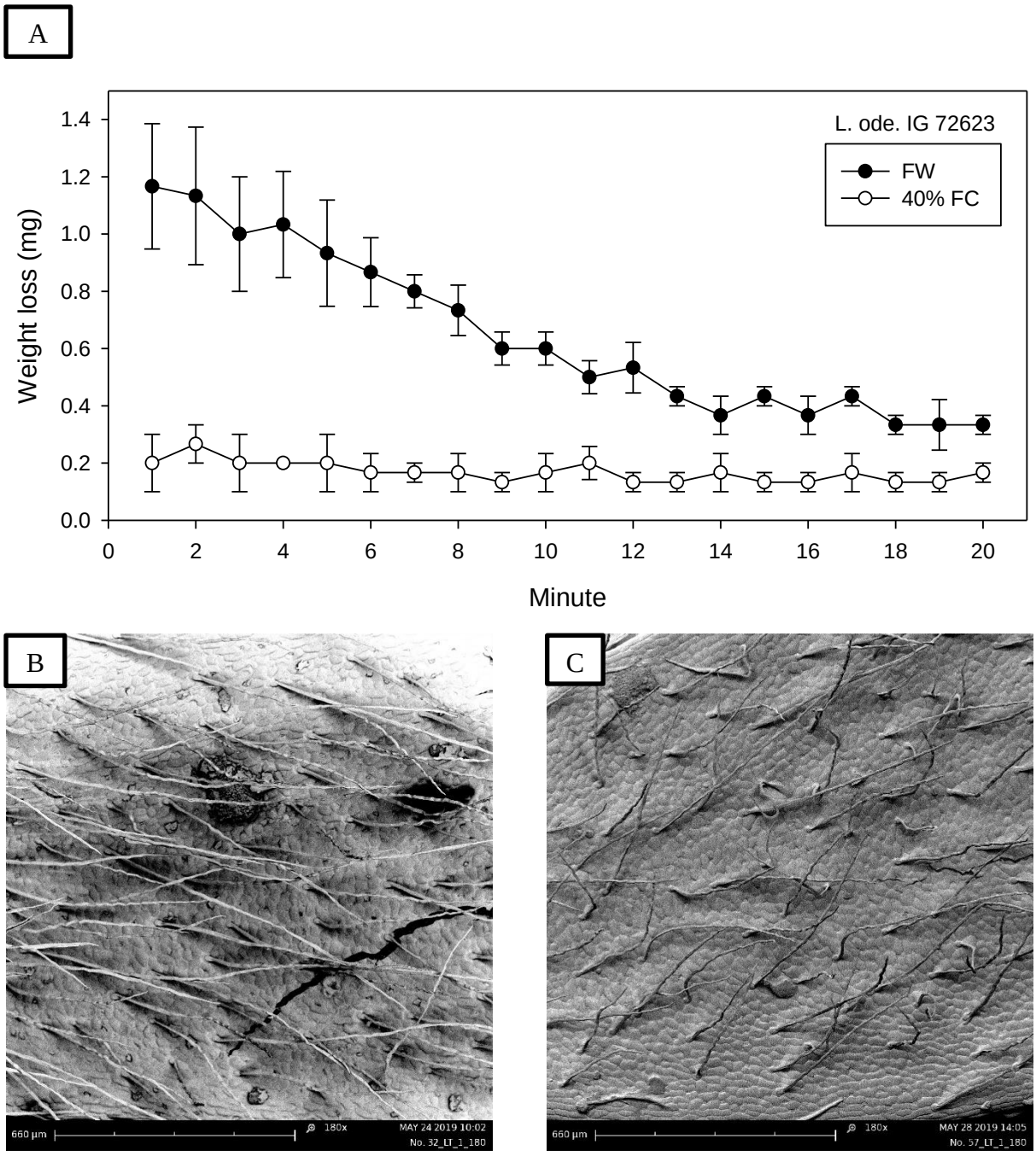


Figure 3.10 (A) Weight loss due to transpiration in *L. ode.* IG 72623 grown under fully watered (FW) and 40% field capacity (40% FC) conditions without adjusting for initial shoot weight and error bars representing standard error of means; and scanning electron microscopy images of adaxial leaf surfaces of *L. ode.* IG 72623 in (B) FW and (C) 40% FC condition at 180x.

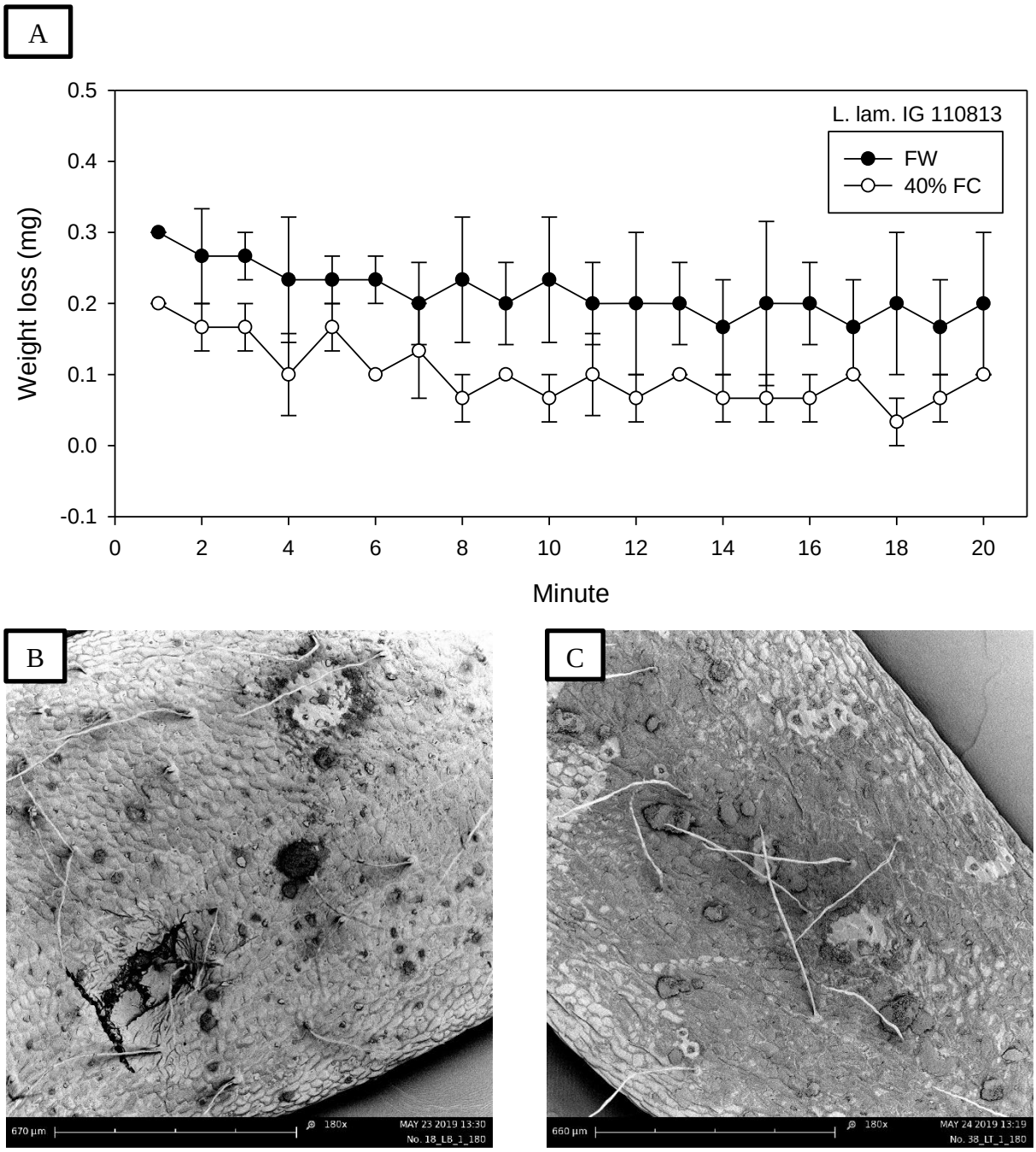


Figure 3.11 (A) Weight loss due to transpiration in *L. lam.* IG 110813 grown under fully watered (FW) and 40% field capacity (40% FC) conditions without adjusting for initial shoot weight and error bars representing standard error of means; and scanning electron microscopy images of adaxial leaf surfaces of *L. lam.* IG 110813 in (B) FW and (C) 40% FC condition at 180x.

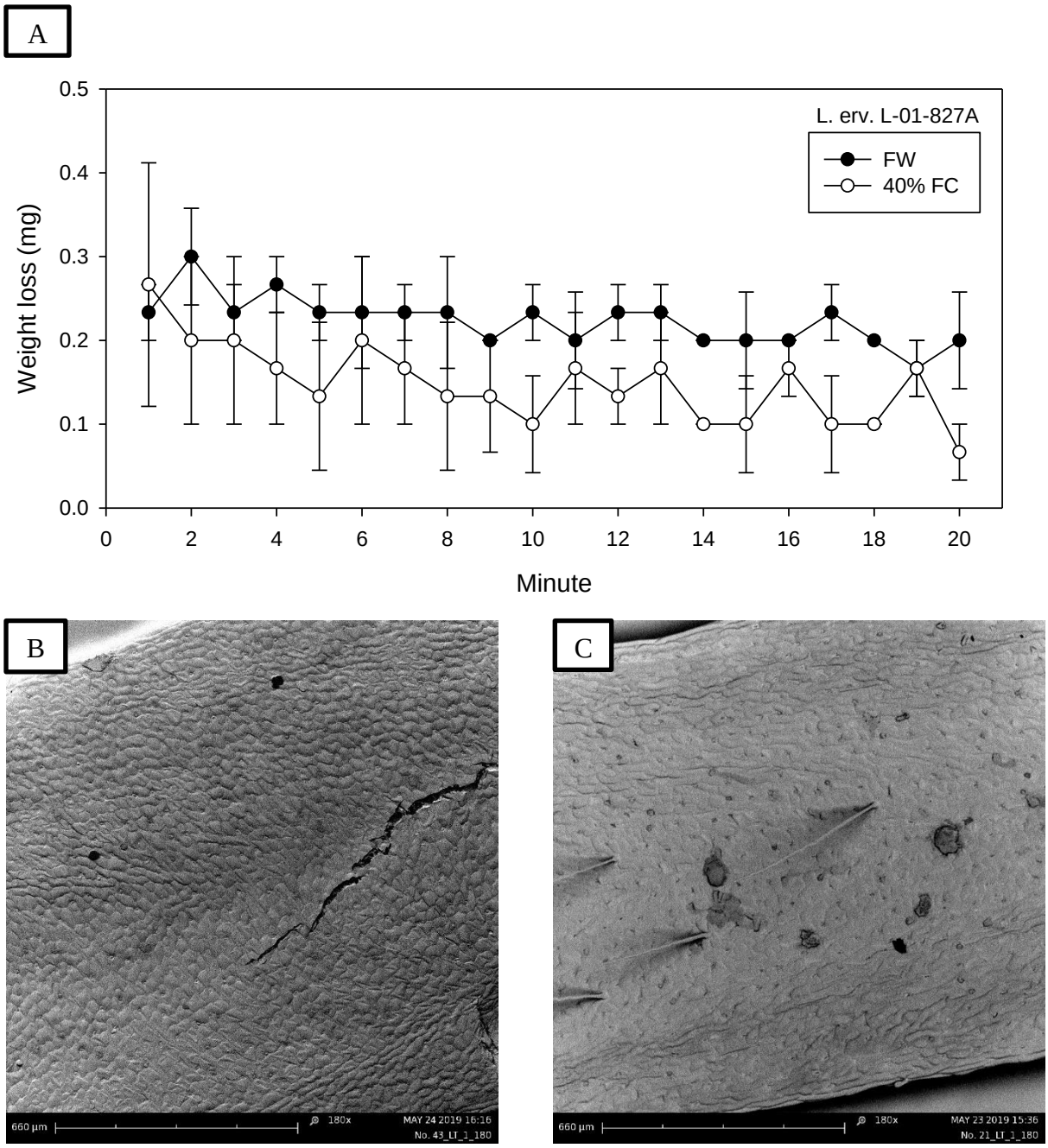


Figure 3.12 (A) Weight loss due to transpiration in *L. erv.* L-01-827A grown under fully watered (FW) and 40% field capacity (40% FC) conditions without adjusting for initial shoot weight and error bars representing standard error of means; and scanning electron microscopy images of adaxial leaf surfaces of *L. erv.* L-01-827A in (B) FW and (C) 40% FC condition at 180x.

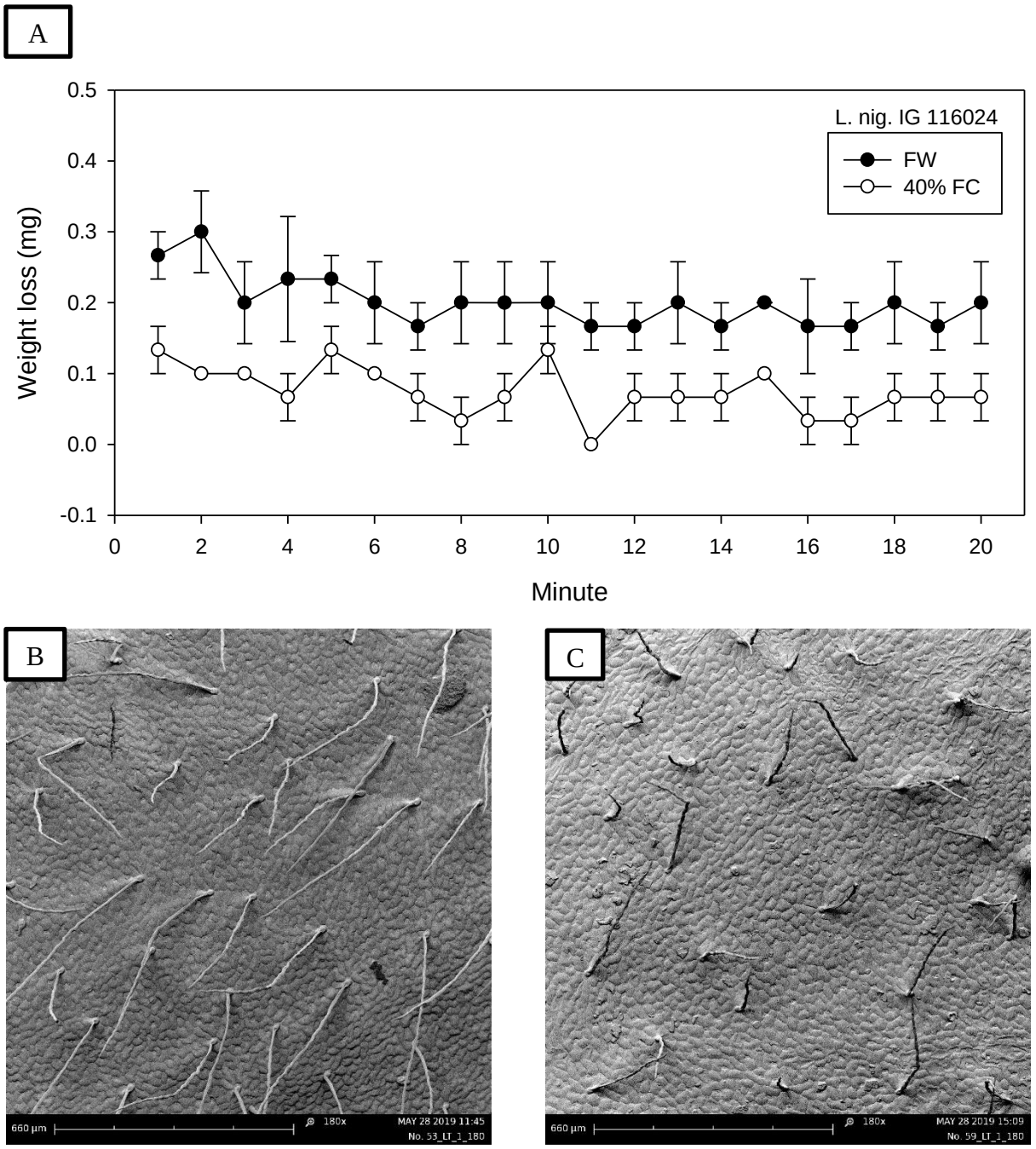


Figure 3.13 (A) Weight loss due to transpiration in *L. nig.* IG 116024 grown under fully watered (FW) and 40% field capacity (40% FC) conditions without adjusting for initial shoot weight and error bars representing standard error of means; and scanning electron microscopy images of adaxial leaf surfaces of *L. nig.* IG 116024 in (B) FW and (C) 40% FC condition at 180x.

Figure 3.14 shows average number of trichomes per mm^2 on adaxial leaf surface of 20 genotypes of *Lens* spp. grown under FW and 40% FC conditions.

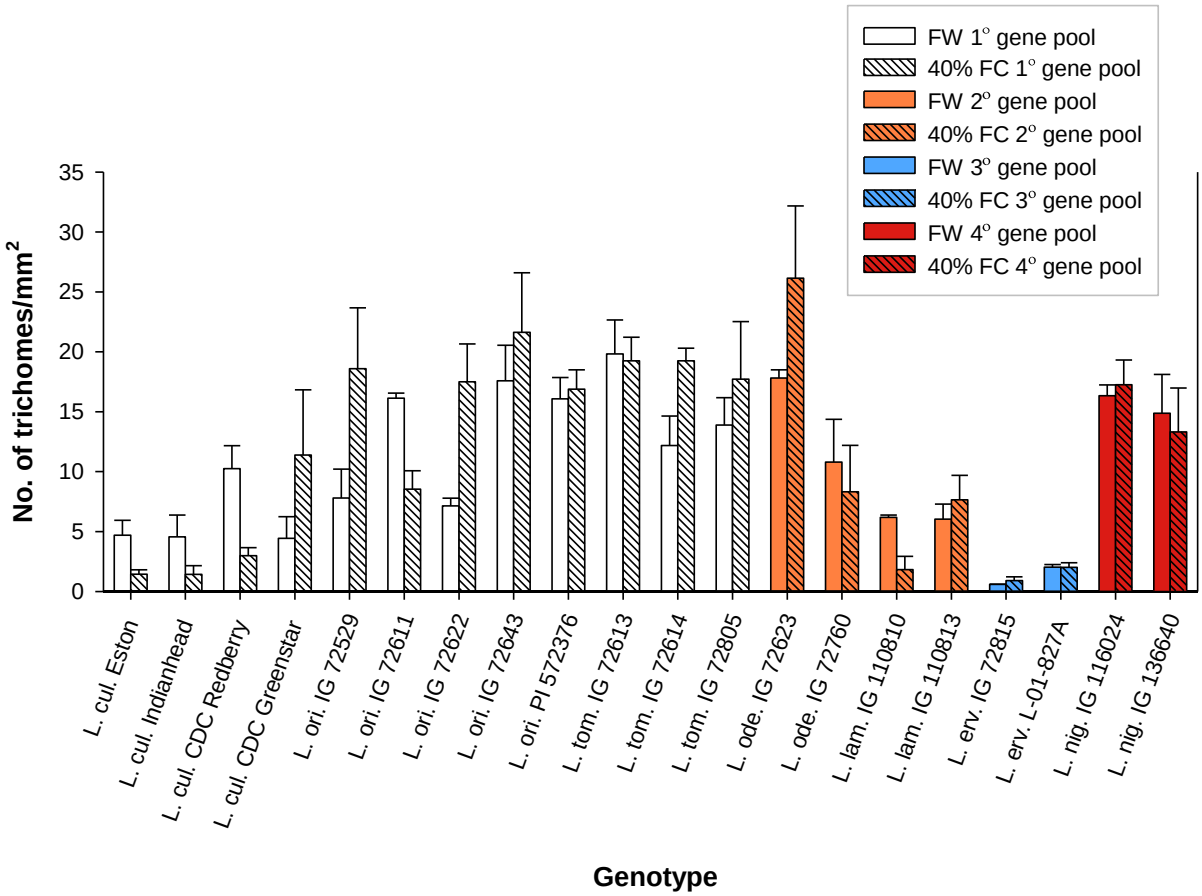


Figure 3.14 Trichome density per unit area of adaxial leaflet surface of wild and cultivated lentil species grown under fully watered (FW) and 40% field capacity (40% FC) conditions. The error bars are standard errors of treatment means.

Analysis of variance done on the fitted model indicated that trichome density per mm^2 did not differ significantly between treatments in the same genotype, but the effect of genotype as well as the effect of interaction between genotype and treatment was significant ($p < 0.05$) (Appendix 2). Additionally, response to drought was not similar between genotypes belonging to the same species. *L. erv.* IG 72815 was observed to have lowest trichome density in both FW and 40% FC conditions, and trichome density was highest in *L. ode.* IG 72623 grown under 40% FC condition, with 17.8 trichomes/mm^2.

Figure 3.15 shows average epidermal cell density per mm^2 on adaxial leaf surface of 20 *Lens* genotypes grown under FW and 40% FC conditions. This provides a measure of difference in epidermal cell size between the wild and cultivated lentil genotypes.

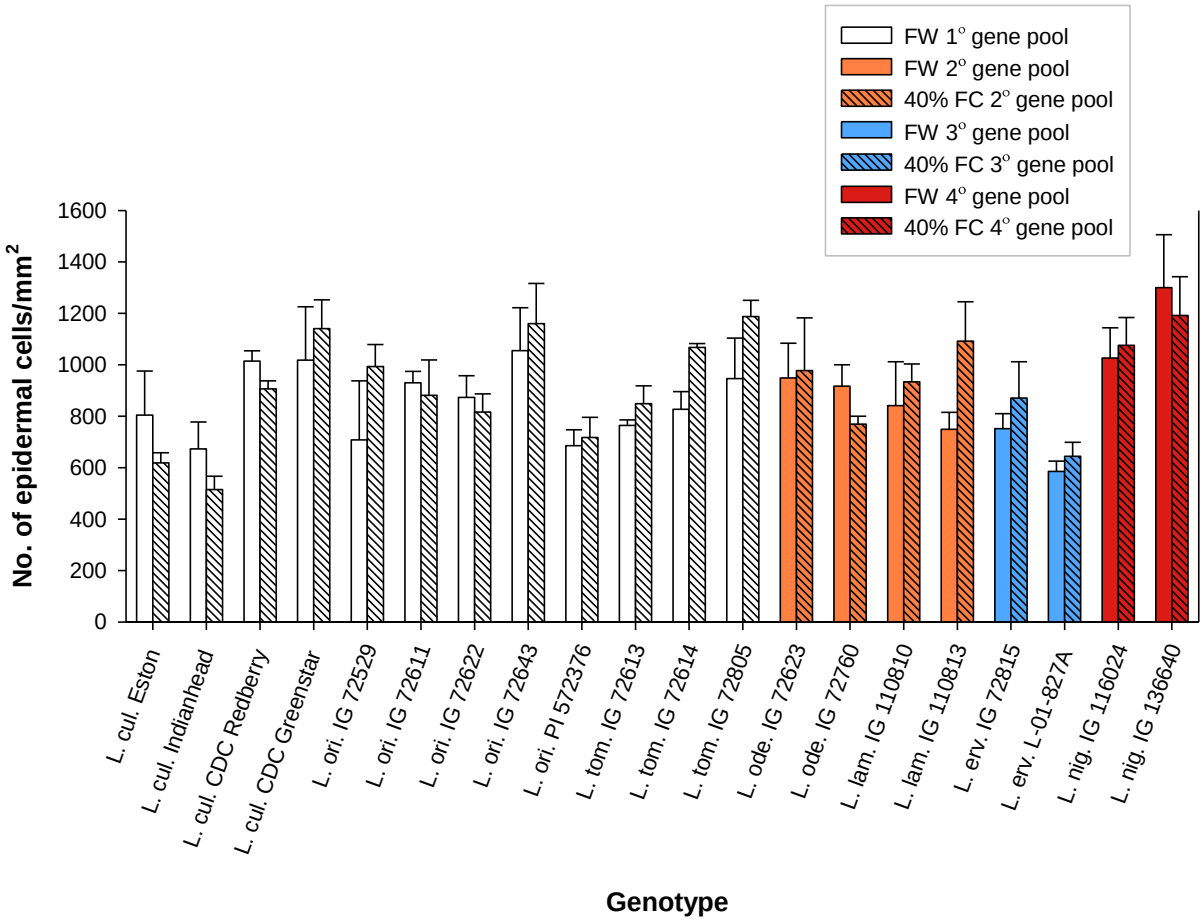


Figure 3.15 Epidermal cell density per unit area of adaxial leaflet surface of wild and cultivated lentil species grown under fully watered (FW) and 40% field capacity (40% FC) conditions. The error bars are standard errors of treatment means.

ANOVA conducted on the fitted model indicated that epidermal cell density did not differ significantly between treatments of the same genotype, and the interaction between genotype and treatment was not significant either (Appendix 3). However, the effect of genotype was significant ($p < 0.001$). Similar observations were made with respect to stomatal index, shown in figure 3.16 and in the ANOVA table in Appendix 4.

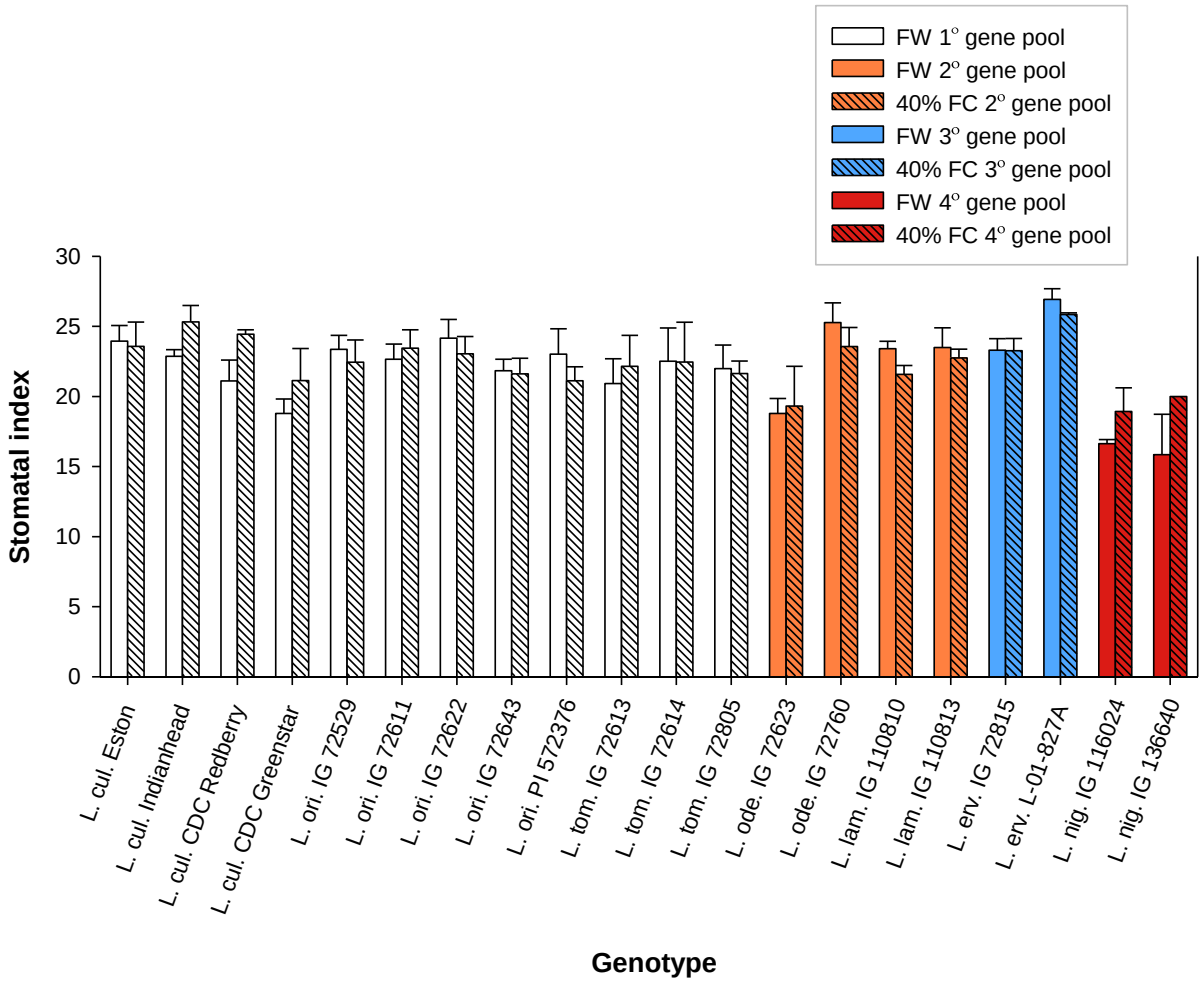


Figure 3.16 Stomatal index of adaxial leaflet surface of wild and cultivated lentil species grown under fully watered (FW) and 40% field capacity (40% FC) conditions. The error bars are standard errors of treatment means.

L. erv. L-01-827A grown under FW condition had highest stomatal index while *L. nig.* IG 136640 grown under FW condition had lowest stomatal index. Stomatal indices remained similar between genotypes when grown under different conditions.

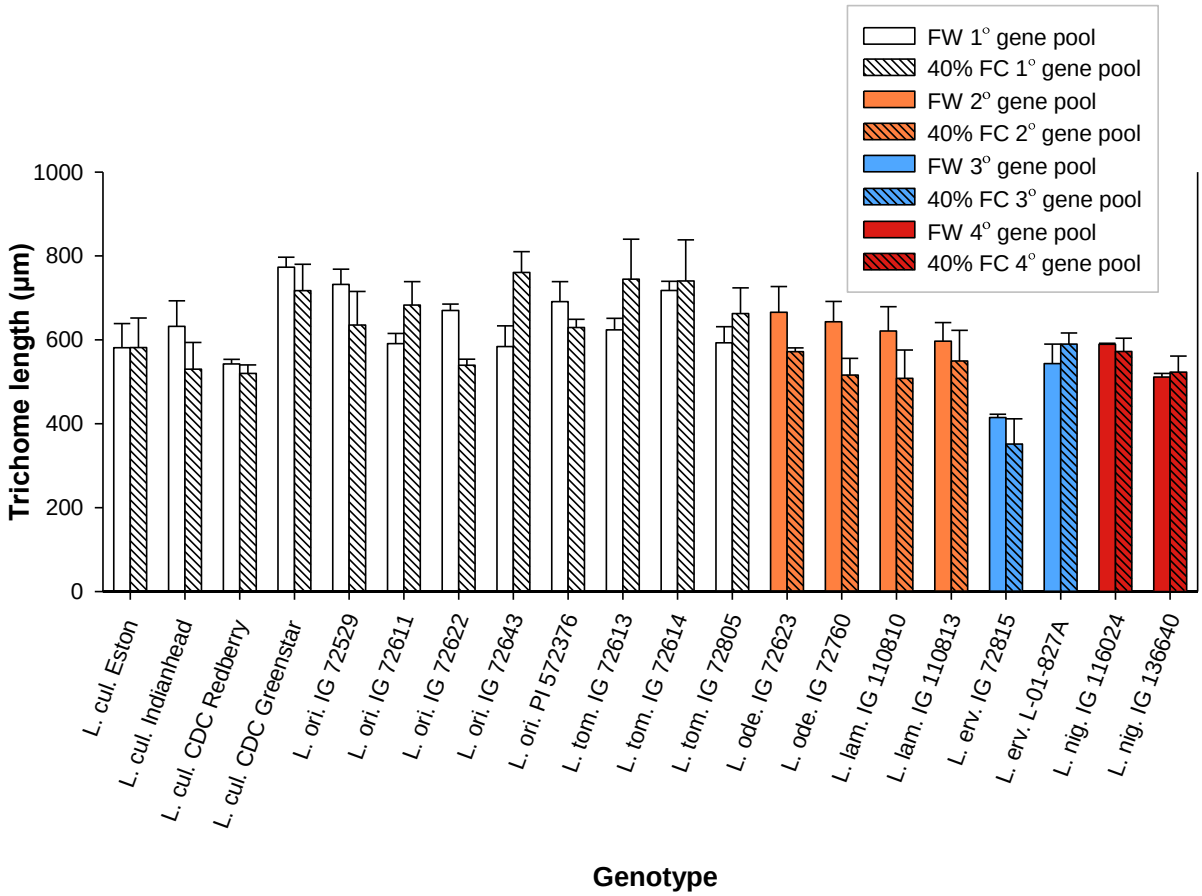


Figure 3.17 Average length of trichomes on adaxial leaflet surface of wild and cultivated lentil species grown under fully watered (FW) and 40% field capacity (40% FC) conditions. The error bars are standard errors of treatment means.

Figure 3.17 shows average trichome length on adaxial leaflet surface of the 20 *Lens* genotypes. *L. erv*. IG 72815 grown under 40% FC condition had shortest trichomes (mean of 352 µm), while *L. cul.* CDC Greenstar grown under FW condition had the longest trichomes (mean of 773 µm). ANOVA on the fitted model indicated no significant effect of treatment or the interaction between genotype and treatment, however, the effect of genotype was significant with $p < 0.001$ (Appendix 5).

Mean values depicted in figures 3.14 to 3.17 are provided in Table 3.2 along with associated standard error values and compact letter displays showing significant differences.

Table 3.2 Means and standard errors of trichome and epidermal cell density, stomatal index, and trichome length on adaxial leaflet surface of 20 *Lens* genotypes grown under fully watered (FW) and 40% field capacity (40% FC) conditions.

Genotype – Treatment	No. of trichomes per mm^2 (SE)	No. of epidermal cells per mm^2 (SE)	Stomatal index	Trichome length (µm)
L. cul. Eston – FW	4.7 (1.2) ^{abcde}	805 (171)^{abc}	23.94 (1.11) ^{abc}	581 (57) ^{abc}
L. cul. Eston – 40% FC	1.4 (0.4) ^{ab}	619 (39)^{ab}	23.57 (1.73) ^{abc}	582 (70) ^{abc}
L. cul. Indianhead – FW	4.6 (1.8) ^{abcde}	673 (104)^{abc}	22.86 (0.48) ^{abc}	632 (61) ^{abc}
L. cul. Indianhead – 40% FC	1.4 (0.7) ^{ab}	515 (52)^{a}	25.32 (1.17) ^{c}	530 (64) ^{abc}
L. cul. CDC Redberry – FW	10.3 (1.9) ^{abcdefg}	1015 (40)^{abc}	21.11 (1.49) ^{abc}	543 (11) ^{abc}
L. cul. CDC Redberry – 40% FC	3.0 (0.7) ^{abcd}	906 (32)^{abc}	24.43 (0.31) ^{bc}	520 (21) ^{abc}
L. cul. CDC Greenstar – FW	4.4 (1.8) ^{abcde}	1018 (207)^{abc}	18.79 (1.03) ^{abc}	773 (24) ^{c}
L. cul. CDC Greenstar – 40% FC	11.4 (5.4) ^{abcdefgh}	1141 (112)^{abc}	21.13 (2.28) ^{abc}	717 (63) ^{c}
L. ori. IG 72529 – FW	7.8 (2.4) ^{abcdefg}	708 (230)^{abc}	23.36 (1.00) ^{abc}	732 (36) ^{c}
L. ori. IG 72529 – 40% FC	18.6 (5.1) ^{efgh}	993 (85)^{abc}	22.44 (1.59) ^{abc}	635 (80) ^{abc}
L. ori. IG 72611 – FW	16.1 (0.4) ^{bcdefgh}	930 (45)^{abc}	22.65 (1.09) ^{abc}	591 (24) ^{abc}
L. ori. IG 72611 – 40% FC	8.5 (1.5) ^{abcdefg}	881 (138)^{abc}	23.45 (1.30) ^{abc}	683 (56) ^{bc}
L. ori. IG 72622 – FW	7.1 (0.6) ^{abcdefg}	873 (85)^{abc}	24.15 (1.34) ^{abc}	670 (15) ^{bc}
L. ori. IG 72622 – 40% FC	17.5 (3.2) ^{defgh}	816 (71)^{abc}	23.04 (1.24) ^{abc}	539 (14) ^{abc}
L. ori. IG 72643 – FW	17.6 (3.0) ^{defgh}	1055 (167)^{abc}	21.83 (0.83) ^{abc}	584 (49) ^{abc}
L. ori. IG 72643 – 40% FC	21.6 (5.0) ^{gh}	1160 (156)^{abc}	21.61 (1.12) ^{abc}	761 (50) ^{c}
L. ori. PI 572376 – FW	16.1 (1.8) ^{bcdefgh}	685 (62)^{abc}	23.02 (1.81) ^{abc}	691 (48) ^{bc}
L. ori. PI 572376 – 40% FC	16.9 (1.6) ^{cdefgh}	718 (78)^{abc}	21.12 (1.00) ^{abc}	629 (20) ^{abc}
L. tom. IG 72613 – FW	19.8 (2.8) ^{fgh}	765 (21)^{abc}	20.92 (1.77) ^{abc}	624 (27) ^{abc}
L. tom. IG 72613 – 40% FC	19.3 (2.0) ^{efgh}	848 (70)^{abc}	22.15 (2.21) ^{abc}	745 (95) ^{c}
L. tom. IG 72614 – FW	12.2 (2.5) ^{abcdefgh}	827 (69)^{abc}	22.51 (2.37) ^{abc}	718 (22) ^{c}
L. tom. IG 72614 – 40% FC	19.3 (1.1) ^{efgh}	1068 (15)^{abc}	22.45 (2.85) ^{abc}	740 (98) ^{c}
L. tom. IG 72805 – FW	13.9 (2.3) ^{abcdefgh}	947 (157)^{abc}	21.99 (1.67) ^{abc}	593 (38) ^{abc}
L. tom. IG 72805 – 40% FC	17.7 (4.8) ^{defgh}	1188 (63)^{abc}	21.63 (0.90) ^{abc}	663 (61) ^{bc}
L. ode. IG 72623 – FW	17.8 (0.7) ^{defgh}	949 (135)^{abc}	18.79 (1.06) ^{abc}	666 (61) ^{bc}
L. ode. IG 72623 – 40% FC	26.1 (6.0) ^{h}	978 (205)^{abc}	19.32 (2.83) ^{abc}	572 (9) ^{abc}
L. ode. IG 72760 – FW	10.8 (3.6) ^{abcdefg}	917 (83)^{abc}	25.27 (1.41) ^{c}	643 (48) ^{bc}
L. ode. IG 72760 – 40% FC	8.3 (3.9) ^{abcdefg}	769 (30)^{abc}	23.56 (1.35) ^{abc}	516 (40) ^{abc}
L. lam. IG 110810 – FW	6.2 (0.2) ^{abcdef}	841 (171)^{abc}	23.41 (0.52) ^{abc}	621 (58) ^{abc}
L. lam. IG 110810 – 40% FC	1.8 (1.1) ^{ab}	934 (70)^{abc}	21.58 (0.63) ^{abc}	508 (68) ^{abc}
L. lam. IG 110813 – FW	6.0 (1.3) ^{abcdef}	749 (66)^{abc}	23.49 (1.40) ^{abc}	597 (44) ^{abc}
L. lam. IG 110813 – 40% FC	7.7 (2.0) ^{abcdefg}	1092 (153)^{abc}	22.75 (0.62) ^{abc}	550 (73) ^{abc}
L. erv. IG 72815 – FW	0.6 (0.0) ^{a}	752 (58)^{abc}	23.3 (0.82) ^{abc}	415 (8) ^{ab}
L. erv. IG 72815 – 40% FC	0.9 (0.3) ^{a}	871 (141)^{abc}	23.27 (0.87) ^{abc}	352 (60) ^{a}
L. erv. L-01-827A – FW	2.0 (0.2) ^{abc}	585 (40)^{ab}	26.92 (0.76) ^{c}	543 (47) ^{abc}
L. erv. L-01-827A – 40% FC	2.0 (0.4) ^{abc}	644 (55)^{abc}	25.85 (0.12) ^{c}	590 (27) ^{abc}
L. nig. IG 116024 – FW	16.3 (0.9) ^{bcdefgh}	1026 (117)^{abc}	16.63 (0.29) ^{ab}	590 (2) ^{abc}
L. nig. IG 116024 – 40% FC	17.3 (2.1) ^{defgh}	1076 (108)^{abc}	18.93 (1.68)^{abc}	572 (32) ^{abc}
L. nig. IG 136640 – FW	14.9 (3.2) ^{abcdefgh}	1300 (205)^{c}	15.85 (2.88) ^{a}	511 (8) ^{abc}
L. nig. IG 136640 – 40% FC	13.3 (3.7) ^{abcdefgh}	1192 (151)^{bc}	20.00 (0.00) ^{abc}	523 (38) ^{abc}

Compact letter display shows significant differences between mean values within columns after performing least square means for multiple comparisons using alpha = 0.05 and adjusting p-value using Tukey method.

3.4 Discussion

Average water loss due to transpiration varied among *Lens* spp. with wild genotypes, especially genotypes of *L. tom.* and *L. ode.*, having higher rates of transpiration compared to cultivated genotypes and *L. nig.* IG 116024 (quaternary gene pool). This suggests that in addition to having been selected for higher yield and other beneficial agronomic traits, lentil cultivars have also been selected for reduced transpiration which may make them better adapted to drought than most of their wild relatives. Plants of most genotypes also had reduced rate of water loss via transpiration when grown under 40% FC compared to plants grown under FW condition. This observed general trend is in agreement with other studies involving wild and cultivated lentil that have found that transpiration rate is higher in FW conditions in both wild and cultivated lentil species (Gorim and Vandenberg, 2017; Rabani, 2018). In this experiment, the only genotypes which had an increased rate of transpiration when grown under 40% FC condition were *L. cul.* CDC Redberry and *L. tom*. IG 72613 (Figure 3.1), and neither of these genotypes have been previously considered in drought studies. However, the rate of transpiration was not significantly different between the two treatments in both genotypes, so the results could be attributed to lack of replication or other human error.

There exists a huge variation in trichome density on the adaxial leaf surface among lentil genotypes, even when grown under FW condition. Except for the genotypes of *L. erv*. and *L. lam*., all other species had at least one genotype that had trichome density similar to *L. tom.* IG 72613, the genotype with highest trichome density when grown under FW condition (Table 3.2). These variations may reflect genotypic influence of adaptation to native microclimate, summarized in Table 3.1. In general, genotypes of *L. cul.*, *L. lam.*, and *L. erv*. had lower trichome density with *L. erv*. IG 72815 having least and shortest trichomes. *L. ori.*, *L. tom.*, *L. ode.*, and *L. nig.* had higher trichome density on their adaxial leaf surface. Previous studies have noted the presence or absence of pubescence on leaflets and pods of *Lens* spp. (Ferguson et al., 2000; Singh et al., 2014; Yildizdoğan et al., 2016). This study confirms these findings and, for the first time, quantifies this variation.

Not all genotypes within the same species reacted to moderate drought in the same manner with respect to regulating their trichome density, epidermal cell density, stomatal index, and trichome length. In the case of *L. cul.*, only CDC Greenstar responded to moderate drought by increasing its trichome density while the other *L. cul.* genotypes decreased the number of

trichomes on their adaxial surface. On the contrary, only *L. ori.* IG 72529 reduced its trichome density under 40% FC condition, while the other *L. ori.* genotypes increased their trichome density. Similar results were observed in the case of other species, where response to drought differed based on each genotype even within the same species. This dynamic response to drought with respect to the regulation of trichome density suggests that other mechanisms might be at play in different genotypes of *Lens* spp. to prevent water loss and to maximize growth under moderate drought. Increase in trichome density due to water deficit conditions has been reported in other studies (Abdulraham and Oladele, 2011; Gonzáles et al., 2008; Nunes et al., 2009), however, cuticular wax layers are also known to protect against water loss in drought conditions (Sánchez et al., 2001; Seo and Park, 2011). Perhaps the genotypes that reduced their trichome numbers increased their production of cuticular waxes to withstand drought, and this needs to be further explored.

While no overall correlation was observed between increase/decrease in trichome density and transpiration water loss, trichome density did appear to be associated with transpiration rate in 11 of the 12 genotypes tested. An increase in trichome density under 40% FC condition was associated with a decrease in transpiration rate and vice versa. The only exception was *L. cul.* Indianhead, where a decrease in trichome density was observed along with a decrease in transpiration rate under 40% FC condition. This genotype has a winter annual growth habit, which could affect its physiological responses in the early life cycle. Another noteworthy case is that of *L. erv*. L-01-827A, which has very few and short trichomes. Its trichome density remained unchanged under moderate drought, yet its transpiration rate was lower under moderate drought compared to FW condition. *L. erv.* is native to the Mediterranean region and is able to thrive in extreme climatic conditions, and thus may be employing other strategies to regulate water loss. In this context, further studies looking into the changes in cuticular waxes of wild and cultivated lentil genotypes are needed to explore the role of waxes in reducing transpiration rate and promoting drought tolerance.

Decreased epidermal cell size or increased epidermal cell density as well as increased stomatal density are other responses to drought stress observed in plants (Bosabalidis and Kofidis, 2002; Shields, 1950). In this study, majority of the genotypes slightly increased epidermal cell density in response to drought, but this response was not observed across all genotypes within the same species except in *L. tom.*, *L. lam.*, and *L. erv.* In *L. cul.*, only CDC Greenstar slightly increased its epidermal cell density, while other cultivars responded to drought by reducing epidermal

cell density and thus increasing epidermal cell size. These changes were not statistically significant and for the most part, epidermal cell density and stomatal index remained similar across genotypes of all species on the adaxial leaf surface.

Not much variation exists with respect to trichome length in *Lens* spp., and trichome length on the adaxial leaf surface did not vary significantly with treatment. This result is in accordance with the results observed in a field study conducted with the perennial herb *Convolvulus chilensis* Pers. (Convolvulaceae) that grows in arid and semiarid environments (Gianoli and González-Teuber, 2005), but available literature associated with this trait seems to vary depending on the plant and species, with studies also reporting increased trichome length in plants grown under drought (Ning et al., 2016; Terletskaya and Kurmanbayeva, 2017).

It should be noted that in this study, only the adaxial leaflet surface was examined. There might be other differences on the abaxial leaf surface that might provide further clues with respect to regulation of water loss in wild and cultivated lentil genotypes. Additionally, cultivated lentil genotypes, especially *L. cul.* CDC Greenstar, have been bred over the years for increased biomass and seed size, etc. The fact that they do not have high trichome density and still have higher biomass and reduced transpiration rate compared to some other wild relatives indicates that there might be other mechanisms that allow them to survive under 40% FC condition. It might be of value to investigate other parameters such as relative leaf water content, stomatal conductance, as well as metabolic aspects such as photosynthetic capture and efficiency and oxidative stress along with the genes and mechanisms associated with these processes (Sánchez-Rodríguez et al., 2010; Xu and Baldocchi, 2003). It might also be worthwhile to look at the effects of heat stress along with water deficit to better understand physiological changes that might occur in lentil during the event of a drought in the Canadian prairies.

3.5 Conclusions

Huge variation exists in transpiration rate as well as leaf surface characteristics within the available lentil germplasm – not only among the wild species, but also among cultivars. This was demonstrated by the dynamic response to drought observed in the wild and cultivated genotypes with respect to regulating transpirational water loss as well as adjusting trichome density, trichome length, epidermal cell density, and stomatal index on their adaxial leaf surface. While most genotypes decreased water loss due to transpiration under moderate

drought, the genotypes *L. cul.* CDC Redberry and *L. ori.* IG 72643 slightly increased their transpiration under moderate drought, albeit the change was not significant. Among the genotypes that reduced their transpiration under moderate drought, a statistically significant response was observed only in the genotype *L. ode.* IG 72623.

Inconsistencies were also observed in genotypic responses to drought with respect to trichomes. While some genotypes predictably increased their trichome density when grown under moderate drought, others decreased it. For most genotypes, an increase in trichome density under moderate drought was associated with a decrease in transpiration rate and vice versa, with the only exception being *L. cul.* Indianhead. *L. cul.* Indianhead has a winter annual growth habit and may thus have an altered physiological response to drought. While the traits of trichome length, epidermal cell density, and stomatal index did not vary much among wild and cultivated genotypes, they were slightly increased or decreased under drought in almost all genotypes. These responses to drought were unique to each genotype and were not species specific, suggesting that the response to drought may be related to the genotypes' native climate at the centre of origin.

In future studies, studying the role of surface waxes and its regulation will likely shed light on how water loss is regulated in genotypes which do not increase their trichome density, epidermal density, or stomatal density when grown under drought. Drought tolerance is a complex trait involving multiple mechanisms, and the variability present in the breeding program can be exploited to breed for drought tolerant cultivars. In order to better model drought on the Canadian prairies, the effects of heat stress should also be considered, and other drought-related metabolic and biochemical processes such as antioxidant activity need to be investigated.

Chapter 4

Effects of trichome coverage on glyphosate efficacy and shoot spray retention in *Lens* spp.

4.1 Introduction

Lentil production and export greatly benefits the Canadian economy and provides a great way to incorporate legumes into crop rotation. Being a nitrogen-fixing pulse crop, lentil reduces the need for N input and benefits the environment along with improving soil quality. However, lentil production is greatly hindered by weeds as lentil is a poor competitor with weeds and there are only a few registered herbicides for use in Canada (Fedoruk and Shirtliffe, 2011). It is thus crucial to breed for characteristics in lentil that render it herbicide resistant.

Glyphosate is one of the most commonly used herbicides in western Canada and it is usually applied in lentil field pre-seeding to clear the field of weeds, and sometimes as a preharvest treatment for late season weed control that also provides desiccation (Beckie et al., 2015). Glyphosate applied preharvest is absorbed by the foliage and translocated to metabolically active parts of the plant, thus helping in crop dry-down (Cessna *et al.*, 2000; Zhang *et al.*, 2016). However, application of glyphosate and other desiccants prior to harvesting might lead to reduction in yield and quality, and leave herbicide residue in the seed exceeding the maximum residue limit (MRL) that renders it unacceptable for export and trade (Zhang *et al.*, 2016; Zhang *et al.*, 2017; Xu *et al.*, 2019).

Herbicide droplet retention on the plant surface and subsequent absorption are affected by various morphological characteristics such as trichomes and cuticular wax, which influence surface wettability and serve as physical barriers (Xu et al., 2011). Trichomes generally reduce herbicide efficacy and optimal surface coverage as spray droplets get suspended on top of the trichomes and prevent them from contacting the epidermal surface (Hess and Falk, 1990). Adjuvants such as surfactants are generally tank-mixed with herbicides to overcome this barrier as surfactants reduce surface tension of herbicide droplets, decrease contact angle with the epidermal surface, and increase spreading of the droplet, thus improving herbicide efficacy (Gerald Young, 2003; Sanyal et al., 2006).

This chapter explores the effect of lentil trichomes on glyphosate efficacy and spray retention in the presence and absence of non-ionic surfactant by the means of lentil genotypes from two different species, *L. culinaris* CDC Redberry and *L. tomentosus* IG 72805, as well as genotypes from interspecific lines arising from the nested association mapping (NAM) population (NAM 38) developed at the CDC using these two genotypes as parents. *L. cul*. CDC Redberry and *L. tom*. IG 72805 were chosen because of their contrasting trichome characteristics on their leaf surfaces. *L. cul.* CDC Redberry is a lentil cultivar with very few observable trichomes on its leaf and pod surfaces, whereas *L. tom.* IG 72805 is a wild lentil genotype with significantly increased trichome density and length on leaf and pod surfaces. NAM 38 sublines segregate genetically for trichome density and length and were used to test a range of trichome characteristics for glyphosate dose-response and spray retention studies. Through the experiments done in this chapter, we aimed to determine if trichomes are a beneficial trait to breed in cultivated lentil with respect to improving herbicide tolerance.

4.2 Materials and methods

All experiments were conducted at the University of Wyoming Agricultural Experiment Station greenhouse in Laramie (41°19'11.4"N, 105°33'29.7"W) from February-April 2019. In all experiments, treatments were applied in a spray chamber equipped with a single, moving, even flat fan nozzle tip (TeeJett 8002E, Spraying Systems Co., Wheaton, IL) calibrated to deliver 187 L/ha of total volume at 276 kPa.

4.2.1 Experiments with *L. cul.* CDC Redberry and *L. tom.* IG 72805

L. cul. CDC Redberry and *L. tom.* IG 72805 seeds were sown on February 19, 2019 in 4-inch square pots containing commercial growth medium (Sunshine SB 300, Sun Gro Horticulture, Vancouver, BC, Canada). Photoperiod was maintained at 16 h day and 8 h night at 20℃ / 22℃ day/night. Plants were watered 2X/day to field capacity and fertilized weekly.

Glyphosate dose-response study with *L. cul.* CDC Redberry and *L. tom.* IG 72805

Experimental design was a completely randomized design with 8 replicates. At 21 d after seeding (DAS), plants were sprayed with Roundup PowerMax (Monsanto Company, St. Louis, Missouri) at seven different doses: 0 g ae/ha (untreated control), 79 g ae/ha, 158 g ae/ha, 336 g ae/ha, 667 g ae/ha, 1334 g ae/ha, and 2669 g ae/ha, and glyphosate formulation was diluted in distilled water. This glyphosate formulation was chosen because its label stated that it can be

mixed with a non-ionic surfactant, although it is not required. Recommended glyphosate rate for desiccation pre-harvest is 900 g ae/ha (Saskatchewan Ministry of Agriculture, 2020).

On 7, 14, and 21 days after treatment (DAT), plants were rated for injury on a percent scale ranging from 0-100%. A rating of 0% injury was given when the plant had no injury and appeared to be like the untreated control, and 100% when the plant was dead. On 21 DAT after taking the injury readings, shoots of all plants were harvested and dried in the incubator at 30℃ and dry shoot biomass was measured using an analytical scale.

To model injury and effect on dry weight after treatment, non-linear regression was performed and a four-parametric log-logistic model (Seefeldt et al., 1995) was used to fit the data using equation (1) using the drc package (Ritz et al., 2015) in R statistical software (R Core Team, 2019).

$$f(x) = c + \frac{(d-c)}{1+\exp\left[b(\log(x)-\log(e)\right]} \quad \text{.....} \quad (1)$$

In equation (1), *b* denotes the slope around point of inflection, *c* and *d* are the lower and upper limits respectively, *e* is ED_{50} or dose eliciting 50% response, and *x* is the rate of glyphosate.

To determine probability of mortality 21 DAT, a binomial dataset was constructed wherein all injury values above 90% were recorded as TRUE for mortality and all values below 90% were recorded as FALSE for mortality. A two-parametric log-logistic model was fit to determine probability of mortality 21 DAT for *L. cul.* CDC Redberry and *L. tom.* IG 72805 with lower and upper limits fixed at 0 and 1 for parameters c and d, respectively in equation (1).

The assumptions of normality and homogeneity of variance were tested with Residuals vs. Fitted and Normal Q-Q plots for all data. For the dry weight data, residuals seemed to be increasing with mean. These data were transformed using Box-Cox transformation which corrected the issue of heterogenous variance and non-normality.

Shoot spray retention assay with *L. cul.* CDC Redberry and *L. tom.* IG 72805

Experimental design was a 2x2 factorial design with 15 replicates. At 37 DAS, 15 plants of each genotype of *L. cul.* CDC Redberry and *L. tom.* IG 72805 were sprayed with the following

solutions (i.e., treatments): (1) Distilled water, and (2) Distilled water + 0.25% v/v non-ionic surfactant Preference (Winfield Solutions, LLC., St. Paul, MN, USA). These treatments are henceforth referred to as 'Water' and 'Water + Surfactant'. Red dye FD&C Red 40 (Spectrum Chemical Mfg. Corp., Gardena, CA, USA) was added to both spray solutions at the concentration of 20 g/L to quantify solution retained on the shoot surface after spraying. Methods described by Kniss and Odero (2013) were followed closely: Once sprayed in the spray chamber, each plant was immediately clipped at the soil surface and placed in a beaker containing 35 mL distilled water using forceps. Plants were washed thoroughly in the beaker to release the retained dye, and the absorbance of the wash solution was quantified at 505 nm using a Genesys 20 spectrophotometer (Geneq Inc., Montreal, QC, Canada). The amount of spray solution retained on the surface of the plant was calculated from a standard curve generated using various concentrations of the red dye (R^2=0.99). Different standard curves were used for the treatments of Water and Water + Surfactant to standardize the effect of surfactant. Plant shoot surface area was measured using an LI-3100C Area Meter (LI-COR Biosciences, Lincoln, Nebraska, USA), and dry weight was measured after incubation of shoot samples at 30℃ for 48 hours. To standardise the amount of spray solution retained per plant, its value was divided by the corresponding value for surface area or dry weight and expressed as µL of shoot spray retention per cm^2 of shoot area (µL/cm^2), and µL of shoot spray retention per g of shoot biomass (µL/g), respectively.

Two-way ANOVA was performed using the interaction of Genotype and Treatment as fixed effects and means between each group were compared using Tukey test with 95% confidence level to identify significant differences between treatments.

4.2.2 Experiments with NAM 38 sublines and parents

The interspecific NAM 38 population was developed from the cross *L. cul.* CDC Redberry x *L. tom.* IG 72805 and performing single seed descent for 7 generations. These NAM 38 sublines were grown in the University of Saskatchewan Agriculture Greenhouse in Saskatoon, Saskatchewan, Canada (52°08'21.3"N, 106°37'54.3"W). When the plants were at the flowering stage, imprints of adaxial surface of youngest fully developed leaflets per plant were taken for each subline using SUMP discs (Sump Laboratory, Tokyo, Japan) as described in the previous chapter. Impressions of leaflet surfaces were visualized via an EVOS FL inverted microscope (Mill Creek, Washington, United States). Three fields of view were captured for each leaflet

sample and trichome density and trichome length were measured using ImageJ (imagej.nih.gov/ij/).

For the experiments in this chapter, a total of 19 NAM 38 sublines (including parents) were selected spanning a range of varying trichome density, length, and coverage (calculated by multiplying trichome density by trichome length, expressed as No. of trichomes/metre) on their leaflet surface. Table 4.1 provides the list of NAM 38 sublines used for dose-response assay and spray retention studies along with their corresponding generation, arranged in the order of least to most trichome coverage on adaxial leaf surface from top to bottom of the list. The parents of the NAM 38 population, *L. cul.* CDC Redberry and *L. tom.* IG 72805, were included in both experiments.

Table 4.1 Leaflet trichome characteristics on adaxial surface of NAM 38 parents and sublines used for dose-response and spray retention studies.

Genotype	Generation	Trichome density (No. of trichomes/mm^2)	Trichome length (µm)	Trichome coverage (No. of trichomes/m)
NAM 38-54^{+}	F7	1.11	404	449
NAM 38-62^{+}	F7	1.56	327	511
***L. cul.* CDC Redberry**$^{*+}$	Parent	1.80	332	597
NAM 38-64^{*+}	F7	1.56	393	614
NAM 38-74*	F7	3.12	314	980
NAM 38-22*	F7	5.00	830	4149
NAM 38-12^{*+}	F7	7.81	675	5273
NAM 38-61^{+}	F7	7.50	816	6120
NAM 38-56*	F7	10.42	711	7406
NAM 38-55*	F7	9.15	1046	9573
NAM 38-48^{+}	F7	15.62	795	12423
NAM 38-4*	F7	25.00	516	12892
NAM 38-102^{+}	F6	18.75	706	13242
NAM 38-107*	F5	25.00	701	17529
NAM 38-103^{*+}	F6	25.00	787	19672
NAM 38-68^{+}	F7	25.00	850	21254
***L. tom.* IG 72805**$^{*+}$	Parent	37.50	670	25124
NAM 38-104*	F6	37.50	679	25457
NAM 38-112^{+}	F6	43.75	710	31084

*Subline used in dose-response study

$^{+}$Subline used in spray retention study

Dose-response and spray retention studies were conducted and analysed the same way as described previously for *L. cul*. CDC Redberry and *L. tom.* IG 72805, except for the following differences:

1. Seeds of NAM 38 sublines were pre-germinated in petri dishes containing 10 µM ABA analog 1019 (developed by Dr. Sue Abrams, patent in process) for 48 h to promote uniform germination and then planted in Ray Leach Cone-tainers that measured 3.8 cm in diameter and 21 cm in depth, with a volume of 164 mL (Stuewe & Sons, Inc., Tangent, Oregon USA), as shown in Figure 4.1.
2. Germinated seeds were planted in Cone-tainers on March 30, 2019 and dose-response study was conducted on April 22, 2019 (23 days after planting (DAP)), and spray retention study was conducted on April 23, 2019 (24 DAP).
3. Greenhouse regime was 25℃ day for 15 h and 18℃ night for 9 h, and humidity was ambient. Plants were watered 2X/day at field capacity.
4. For the dose-response study, experimental design was a completely randomized design with 7 replicates. Plants were sprayed with Roundup PowerMax (Monsanto Company, St. Louis, Missouri) at the following eight doses: 0 g ae/ha (untreated control), 42 g ae/ha, 168 g ae/ha, 334 g ae/ha, 670 g ae/ha, 1330 g ae/ha, 2670 g ae/ha, and 5340 g ae/ha, with glyphosate formulation diluted in distilled water.
5. For spray retention study, 15 reps/treatment were used for the following NAM 38 sublines: NAM 38-48, NAM 38-54, NAM 38-61, NAM 38-62, NAM 38-68, NAM 38-102, NAM 38-112, *L. cul.* CDC Redberry, and *L. tom.* IG 72805; 10 reps/treatment were used for NAM 38-64 and NAM 38-103; and 9 reps/treatment were used for NAM 38-12. For data analysis, linear models were fit, and ANOVA was conducted using shoot spray retention as the continuous response variable and treatment, genotype, and their interaction as explanatory variables. To visualize patterns in the scatterplot, a line of best fit was drawn using the geom_smooth function and the method “lm” in the package ggplot2 in R (Wickham, 2016).

Figure 4.1 Some NAM 38 sublines planted in Ray Leach Cone-tainers

4.3 Results

4.3.1 Dose-response assay with *L. cul.* CDC Redberry and *L. tom.* IG 72805

A four-parametric log-logistic model was fit to describe % injury 21 DAT for *L. cul.* CDC Redberry and *L. tom.* IG 72805. Figure 4.2 shows the response curve for injury 21 DAT as a function of glyphosate rate ranging from 0-2669 g ae/ha. Recommended glyphosate rate for desiccation pre-harvest is 900 g ae/ha. Parameter values for the curve in Figure 4.2 along with standard errors are shown in Table 4.2. In the model, parameter d, or the upper limit for injury was set to 100, indicating 100% injury or dead plant. Based on the lack of fit test, the model adequately described the response data ($p = 0.285$). It was observed that injury 21 DAT in *L. cul.* CDC Redberry was consistently higher than *L. tom.* IG 72805 at all rates of glyphosate (Figure 4.2). Comparison of ED_{50} values for injury at 21 DAT show a 3-fold difference between *L. cul.* CDC Redberry and *L. tom.* IG 72805, implying that 3 times more glyphosate is required to cause 50% injury in *L. tom.* IG 72805 compared to *L. cul.* CDC Redberry (Table 4.2). This difference in ED_{50} values was significant with p-value <0.001 using robust standard errors using Sandwich estimators.

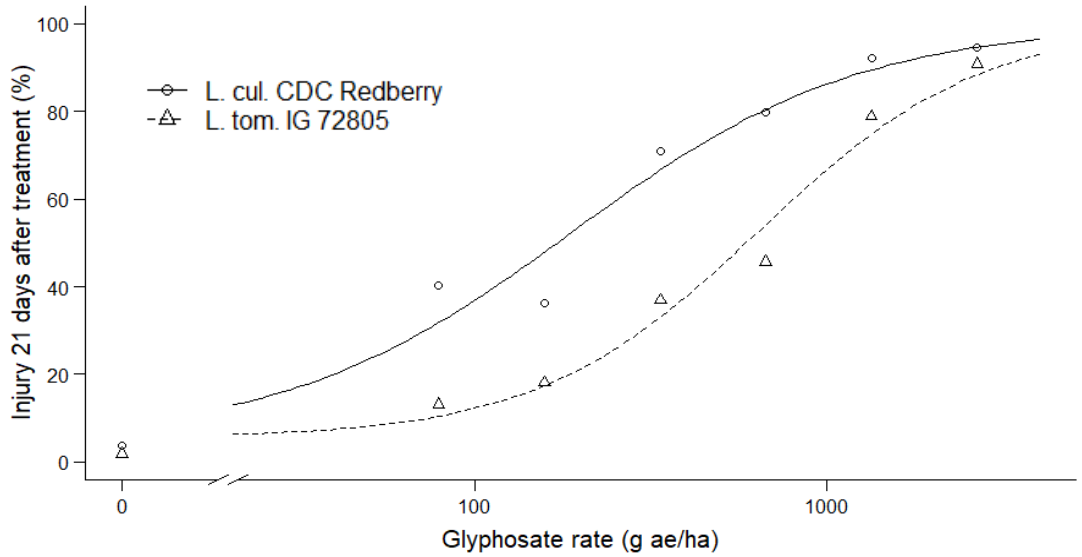


Figure 4.2 % Injury at 21 days after spraying *L. cul.* CDC Redberry and *L. tom.* IG 72805 with glyphosate doses ranging from 0-2669 g ae/ha.

Table 4.2 Parameter estimates and standard errors (SE) from four-parameter log-logistic model of % injury 21 DAT of *L. cul.* CDC Redberry and *L. tom.* IG 72805 treated with glyphosate doses ranging from 0-2669 g ae/ha. b-slope, c-lower limit, d-upper limit (fixed at 100), e-ED_{50} or dose eliciting 50% response.

	b (SE)	**c (SE)**	**d (SE)**	**e (SE)**
L. cul. CDC Redberry	-1.064 (0.331)	4.688 (7.198)	100 (9.891)	188.12^{a} (59.023)
L. tom. IG 72805	-1.376 (0.445)	5.447 (6.559)	100 (14.461)	641.306^{b} (196.91)

To determine probability of mortality 21 DAT, a binomial dataset was fit using a four-parametric log-logistic model with lower and upper limits fixed at 0 and 1, respectively. Lack of fit test determined the p-value to be 0.804, indicating that the model adequately fit the response data. Probability of mortality 21 DAT for *L. cul.* CDC Redberry was higher than that of *L. tom.* IG 72805 for almost all doses except close to the most extreme dose of 2669 g ae/ha (Figure 4.3).

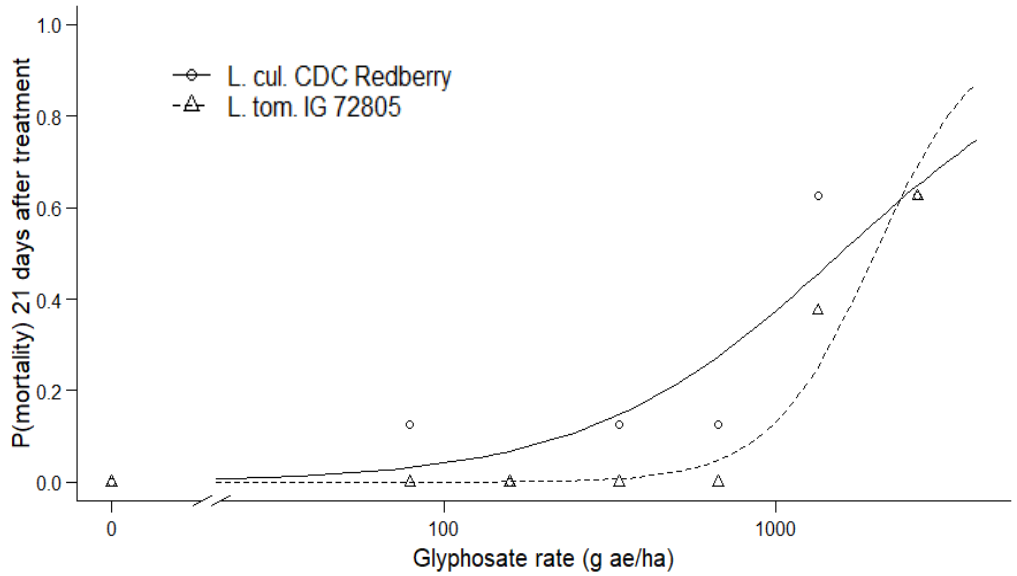


Figure 4.3 Probability of mortality at 21 days after spraying *L. cul.* CDC Redberry and *L. tom.* IG 72805 with glyphosate doses ranging from 0-2669 g ae/ha.

Similar to the model used for describing injury at 21 DAT, a four-parametric log-logistic model was used to depict above ground dry biomass 21 DAT in *L. cul.* CDC Redberry and *L. tom.* IG 72805. Non-normality/heterogeneity in the model was adjusted through optimal Box-Cox transformation, and the p-value after performing the lack of fit test was 0.397, indicating that the model adequately described the response data (Figure 4.4).

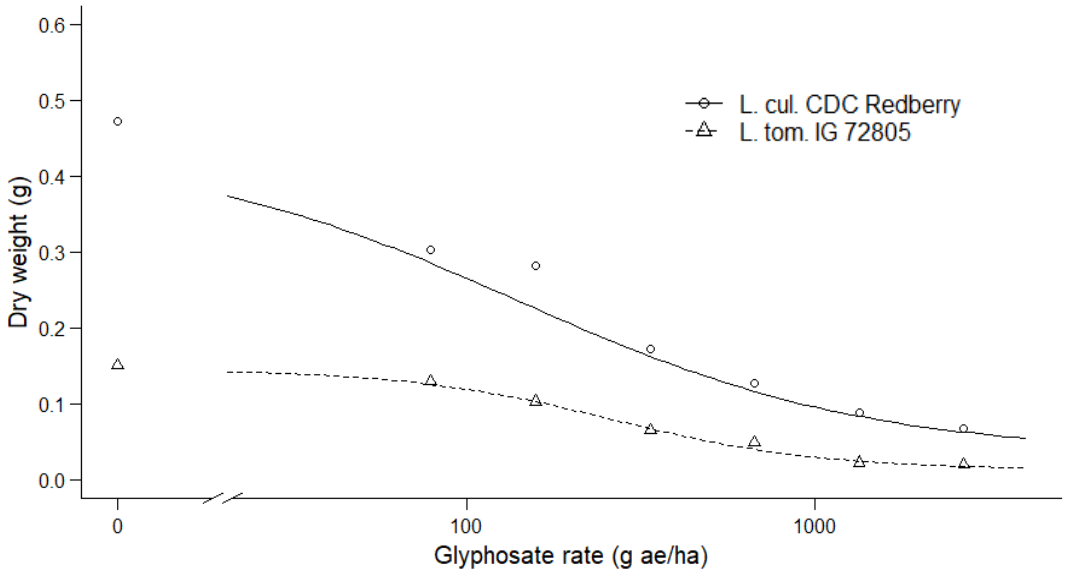


Figure 4.4 Responses of *L. cul.* CDC Redberry and *L. tom.* IG 72805 to Roundup PowerMax (glyphosate rate ranging from 0-2669 g ae/ha) expressed as a function of shoot dry mass 21 days after application.

Table 4.3 shows the values for parameters b, c, d, and e in the model used in Figure 4.4 along with their standard errors. There was approximately 2-fold difference in the ED_{50} values of *L. tom.* IG 72805 and *L. cul.* CDC Redberry, meaning that about 2 times more glyphosate was required to reduce shoot dry weight by 50% in the case of *L. tom.* IG 72805 when compared to *L. cul.* CDC Redberry. This difference in ED_{50} values was significant with p-value <0.001 using robust standard errors using Sandwich estimators.

Table 4.3 Parameter estimates and standard errors (SE) from four-parameter log-logistic model of dry weight of *L. cul.* CDC Redberry and *L. tom.* IG 72805 treated with glyphosate doses ranging from 0-2669 g ae/ha. b-slope, c-lower limit, d-upper limit, e-ED_{50} or dose eliciting 50% response.

	b (SE)	**c (SE)**	**d (SE)**	**e (SE)**
L. cul. CDC Redberry	0.869 (0.324)	0.034 (0.031)	0.438 (0.066)	140.05[a] (61.106)
L. tom. IG 72805	1.453 (0.390)	0.013 (0.005)	0.146 (0.019)	261.49[b] (74.493)

4.3.2 Shoot spray retention study with *L. cul.* CDC Redberry and *L. tom.* IG 72805

Figure 4.5 shows differences in shoot spray retention between *L. cul.* CDC Redberry and *L. tom.* IG 72805 when sprayed with solutions containing water and water + non-ionic surfactant. ANOVA of spray retention showed that the interaction between genotype and treatment was significant (p <0.0001) when spray retention was measured as a function of shoot area as well as shoot biomass. ANOVA tables can be found in Appendices 8 and 9.

Post-hoc testing using Tukey's HSD revealed that *L tom.* IG 72805 had significantly higher spray retention compared to *L. cul.* CDC Redberry when sprayed with only water. In the case of *L. cul.* CDC Redberry mean shoot spray retention values (per unit shoot area) were 0.76 uL/cm^2 and 0.90 uL/cm^2 for water and water + surfactant treatments, respectively. Thus, addition of surfactant increased shoot spray retention. However, in the case of *L. tom.* IG 72805 shoot spray retention decreased with the addition of surfactant, with mean retention going down from 0.86 uL/cm^2 when sprayed with water to 0.79 uL/cm^2 when sprayed with water + surfactant. Although addition of surfactant significantly increased spray retention in *L. cul.* CDC Redberry, the drop in spray retention after the addition of surfactant in the case of *L. tom.* IG 72805 was not statistically significant. A similar trend was observed when comparing spray retention per unit shoot dry weight between the two genotypes (Figure 4.5 B).

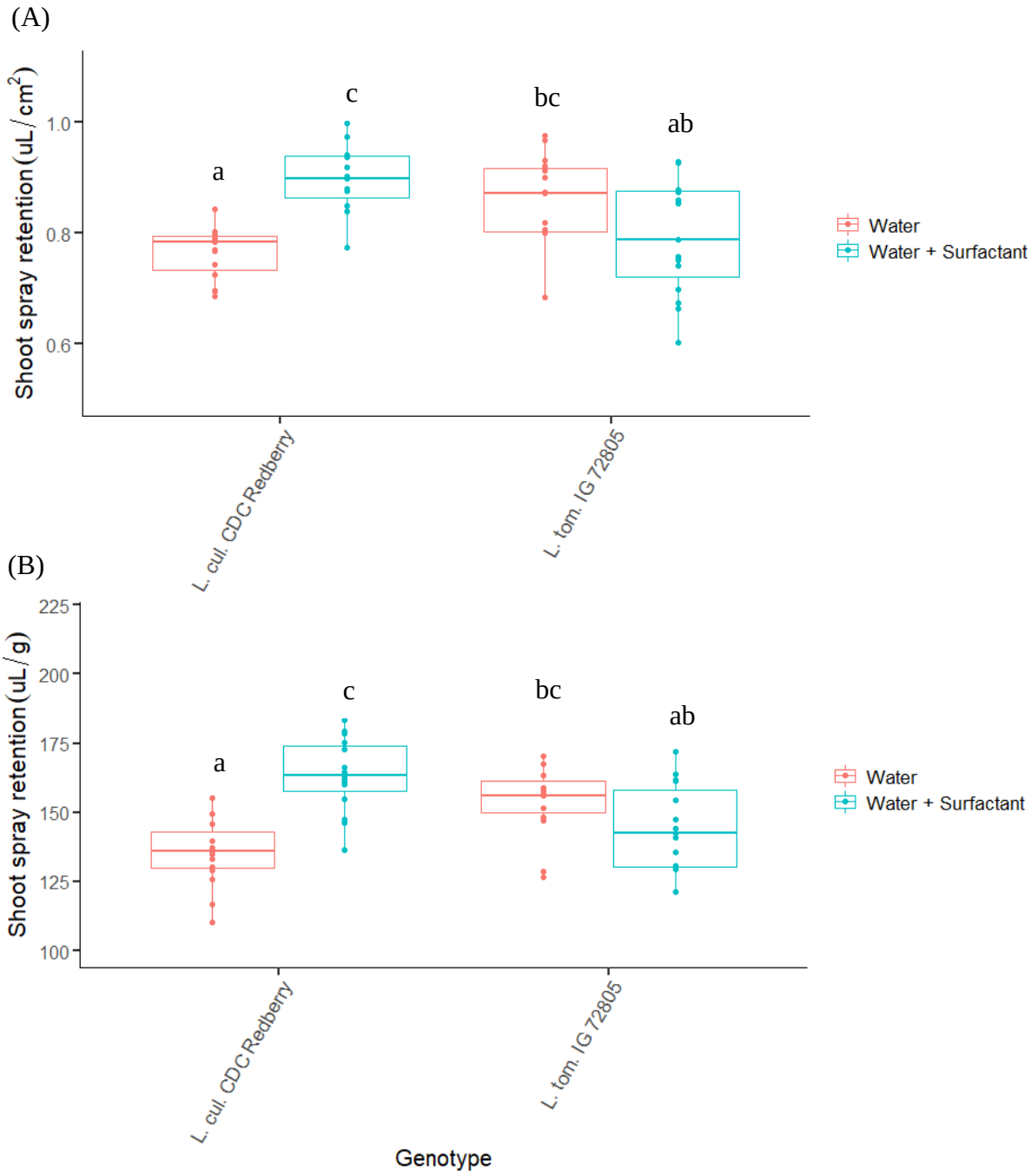


Figure 4.5 Shoot spray retention (A) per unit area and (B) per unit dry weight of *L. cul.* CDC Redberry and *L. tom.* IG 72805 after spraying with Water and Water + Surfactant solutions. Compact letter displays show significant difference between treatment means after performing Tukey's test using alpha = 0.05.

4.3.3 Shoot spray retention study with NAM 38 parents and sublines

Spray retention with NAM 38 lines showed a significant impact of treatment, genotype, and their interaction both in the case of spray retention per unit area and spray retention per unit dry weight. ANOVA tables with test statistic and p-value for each explanatory variable can be found in Appendices 10 and 11. Table 4.4 shows mean shoot spray retention after spraying NAM 38 sublines with water and water + surfactant, and Figures 4.6, 4.7, and 4.8 show the change in retention with increasing trichome density, length, and coverage, respectively.

Table 4.4 Mean spray retention per unit shoot area and per unit shoot dry weight of NAM 38 sublines. Genotypes are arranged in order of increasing trichome coverage.

Genotype	Trichome coverage (No. of trichomes/m)	Treatment	Shoot spray retention ($\mu L/cm^2$)	Shoot spray retention ($\mu L/g$)
NAM 38-54	449	Water	1.06^{ae}	227^{su}
		Water + Surfactant	1.11^{aef}	209^{rsu}
NAM 38-62	511	Water	1.54^{f}	265^{u}
		Water + Surfactant	1.42^{ef}	251^{u}
***L. cul.* CDC Redberry**	597	Water	1.18^{bdef}	271^{u}
		Water + Surfactant	1.35^{ef}	266^{u}
NAM 38-64	614	Water	1.25^{bdef}	242^{su}
		Water + Surfactant	1.45^{ef}	251^{tu}
NAM 38-12	5273	Water	1.22^{bdef}	259^{tu}
		Water + Surfactant	1.17^{aef}	234^{su}
NAM 38-61	6120	Water	1.28^{def}	248^{tu}
		Water + Surfactant	1.16^{bde}	250^{tu}
NAM 38-48	12423	Water	0.95^{ad}	243^{tu}
		Water + Surfactant	1.06^{ae}	230^{su}
NAM 38-102	13242	Water	1.30^{def}	274^{u}
		Water + Surfactant	0.93^{ad}	185^{qst}
NAM 38-103	19672	Water	1.30^{bdef}	234^{su}
		Water + Surfactant	0.91^{acd}	139^{qr}
NAM 38-68	21254	Water	1.32^{cef}	245^{tu}
		Water + Surfactant	1.20^{bdef}	222^{su}
***L. tom.* IG 72805**	25124	Water	1.30^{def}	235^{su}
		Water + Surfactant	0.76^{a}	131^{q}
NAM 38-112	31084	Water	1.14^{bde}	241^{tu}
		Water + Surfactant	0.90^{ab}	171^{qs}

Compact letter display shows significant differences between treatment means after Tukey's HSD test using alpha = 0.05. Means were compared separately for shoot spray retention per unit area and shoot spray retention per unit dry weight.

Upon post-hoc testing of spray retention per unit area among NAM 38 lines, a significant difference between treatments was only observed in the case of *L. tom*. IG 72805, where spray retention significantly decreased after the addition of the surfactant (Table 4.4). When comparing shoot spray retention per unit dry weight, significant differences between treatments are observed in the case of NAM 38-102, NAM 38-103, *L. tom*. IG 72805, and NAM 38-112 (Table 4.4), which have high trichome coverage ranging from 13242 trichomes/m to 31084 trichomes/m (Table 4.1).

Increasing trichome density on the leaf surface did not have much effect on shoot spray retention when sprayed only with water, as retention remained almost constant; However, spray retention greatly decreased with the addition of surfactant (Figure 4.6 A and B). Similar trends were observed when comparing the effects of leaf trichome length and trichome coverage on spray retention, i.e., adding surfactant led to a decrease in spray retention as trichome length and coverage increased (Figures 4.7 and 4.8).

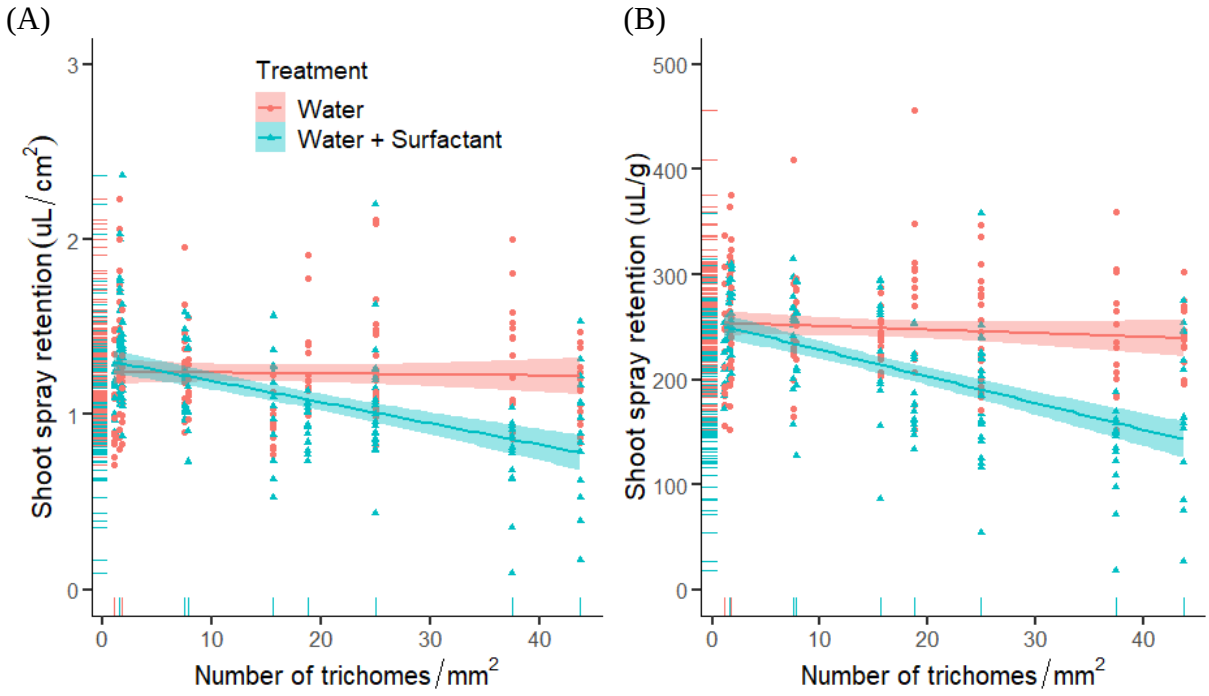


Figure 4.6 Shoot spray retention (A) per unit shoot area and (B) per unit shoot dry weight as a function of trichome density after spraying 12 NAM 38 sublines with Water and Water + Surfactant solutions.

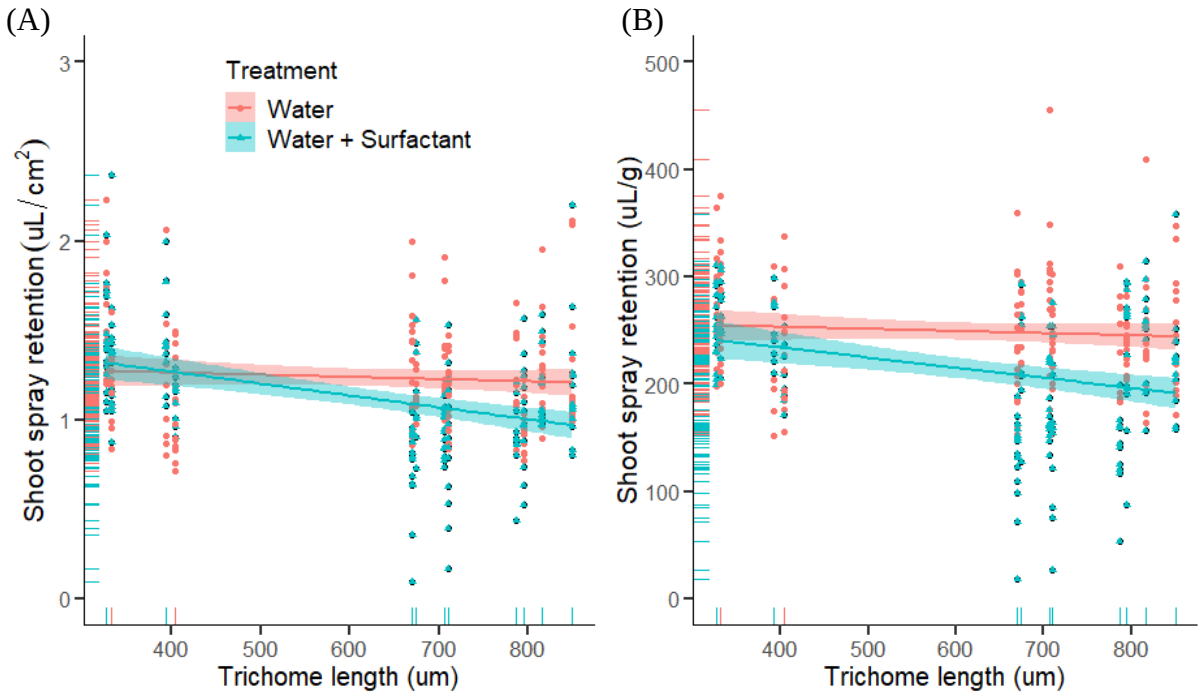


Figure 4.7 Shoot spray retention (A) per unit shoot area and (B) per unit shoot dry weight as a function of trichome length after spraying 12 NAM 38 sublines with Water and Water + Surfactant solutions.

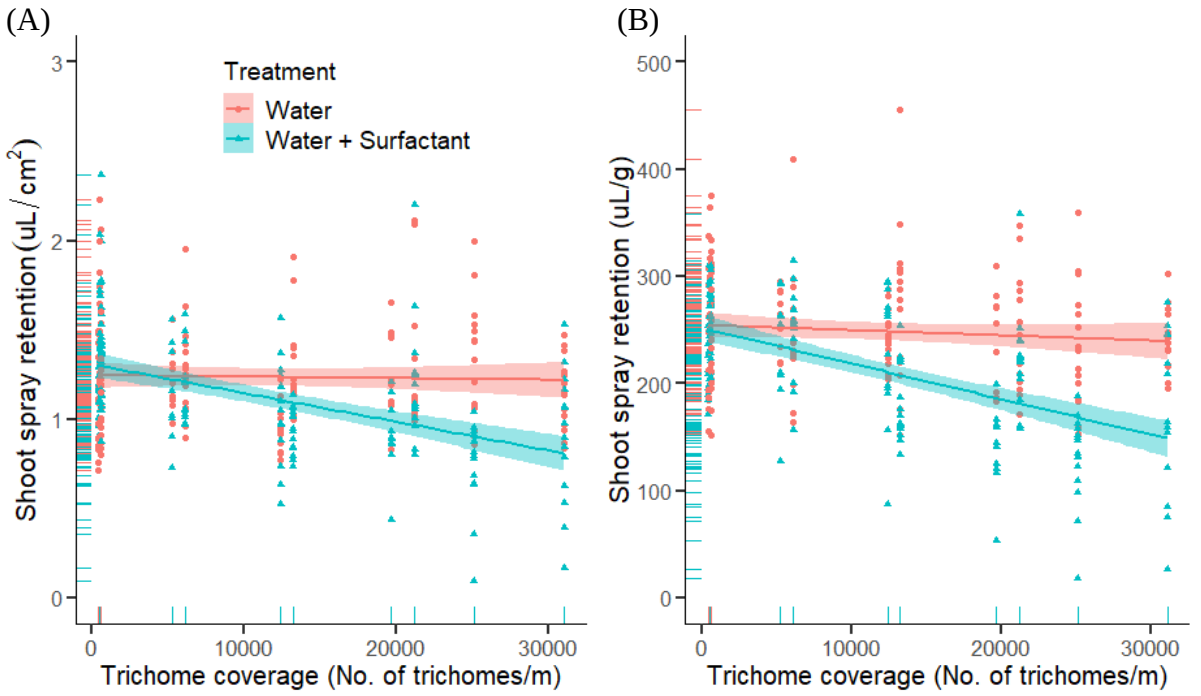


Figure 4.8 Shoot spray retention (A) per unit shoot area and (B) per unit shoot dry weight as a function of trichome coverage (trichome density x trichome length) after spraying 12 NAM 38 sublines with Water and Water + Surfactant solutions.

4.3.4 Dose-response study with NAM 38 parents and sublines

Dose-response experiment evaluating the response of 12 NAM 38 sublines (including parents) after spraying with varying doses of glyphosate ranging from 0-5340 g ae/ha was conducted. Here, results are shown for 6 of the 12 genotypes. The chosen genotypes represent a subset of the full range in trichome density, length, and coverage. Full results and analyses including all 12 genotypes are found in Appendices 12-16.

A four-parametric log-logistic model was fit to indicate % injury 21 DAT in the NAM 38 sublines (Figure 4.9, parameter values along with standard errors are shown in Table 4.5). Parameter d, or the upper limit for injury was set to 100, indicating 100% injury or dead plant. Based on the lack of fit test, the model adequately described the response data ($p = 0.926$). NAM 38-22 had least injury at every dose (Figure 4.9), as reflected in it having the highest ED_{50} value of 2043.17 g ae/ha (Table 4.5). Lowest ED_{50} value was observed in NAM 38-103, with an ED_{50} of 1278.78 g ae/ha (Table 4.5). There were no statistically significant differences between ED_{50} values for injury.

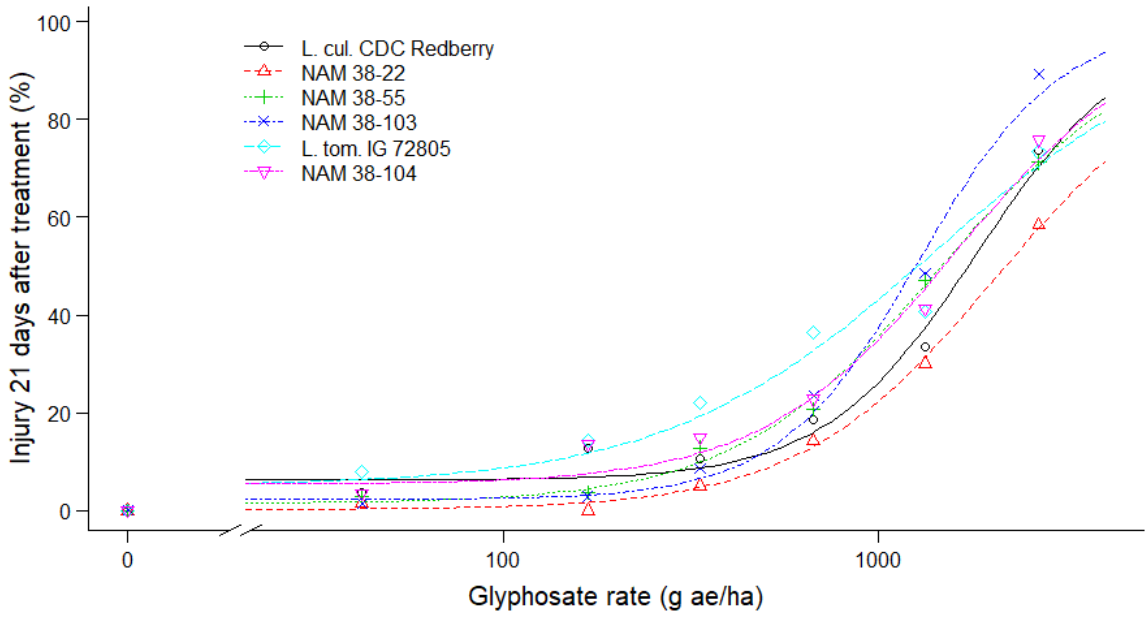


Figure 4.9 Injury at 21 days after spraying NAM 38 sublines with glyphosate dose ranging from 0-5340 g ae/ha. Genotypes in the legend are arranged in order of least to most trichome coverage on the leaf.

Table 4.5 Parameter estimates and standard errors (SE) from four-parameter log-logistic model of % injury 21 days after treatment of 6 NAM 38 sublines with glyphosate doses ranging from 0-5340 g ae/ha. b-slope, c-lower limit, d-upper limit (fixed at 100), e-ED_{50} or dose eliciting 50% response.

	b (SE)	c (SE)	d (SE)	e (SE)
L. cul. CDC Redberry	-2.137 (0.911)	6.271 (3.741)	99.003 (15.649)	1826.182 (380.285)
NAM 38-22	-1.656 (0.612)	0.204 (3.698)	94.764 (22.16)	2043.172 (741.762)
NAM 38-55	-1.591 (0.527)	1.468 (4.031)	97.581 (15.675)	1451.647 (399.943)
NAM 38-103	-2.318 (0.55)	2.297 (3.508)	100 (7.149)	1278.785 (155.717)
L. tom. IG 72805	-1.22 (0.308)	5.052 (4.739)	100 (13.265)	1386.843 (414.948)
NAM 38-104	-1.674 (0.628)	5.381 (4.308)	100 (15.393)	1598.099 (387.96)

To model above ground dry biomass 21 DAT in the NAM 38 sublines, another four-parametric log-logistic model was used. Non-normality/heterogeneity in the model was adjusted through optimal Box-Cox transformation, and the p-value after performing the lack of fit test was 0.303, indicating that the model adequately described the response data (Figure 4.10). Table 4.6 shows the values for parameters b, c, d, and e in the model used in Figure 4.10 along with their standard errors. *L. cul*. CDC Redberry had the highest ED_{50} value of 802.41 g ae/ha and *L. tom.* IG 72805 had the lowest ED_{50} of 115.95 g ae/ha, indicating that about 7 times more glyphosate was required to reduce dry weight of *L. cul.* CDC Redberry by 50% compared to *L. tom*. IG 72805 (Table 4.6).

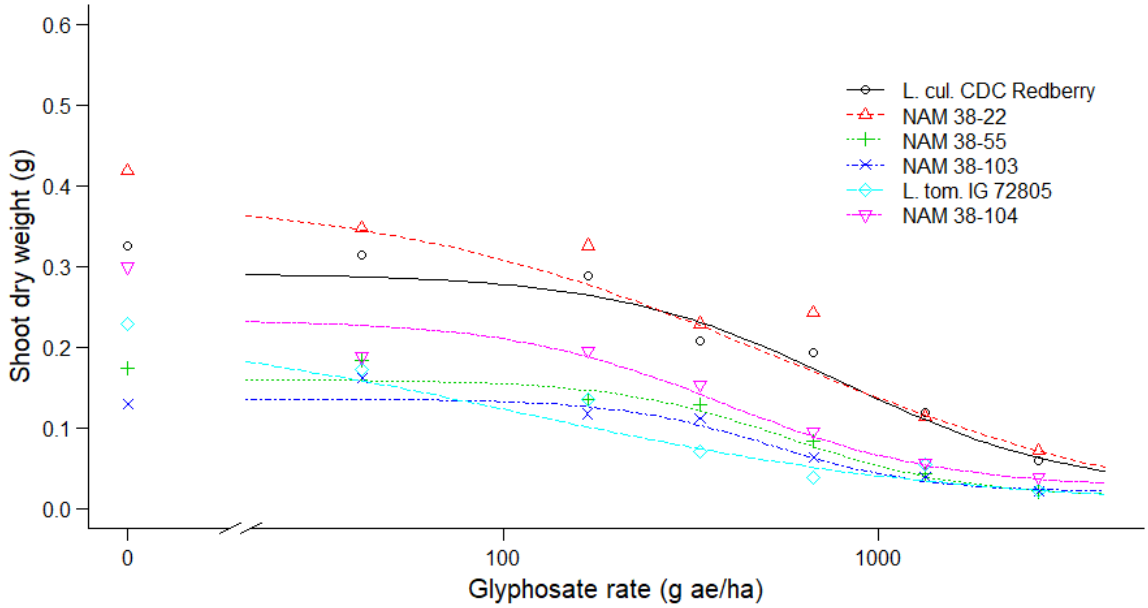


Figure 4.10 Responses of NAM 38 sublines to glyphosate rate ranging from 0-5340 g ae/ha expressed as a function of shoot dry mass 21 days after application. Genotypes in the legend are arranged in order of least to most trichome coverage on the leaf.

Table 4.6 Parameter estimates and standard errors (SE) from four-parameter log-logistic model of dry weight of 6 NAM 38 sublines treated with glyphosate doses ranging from 0-5340 g ae/ha. b-slope, c-lower limit, d-upper limit, e-ED_{50} or dose eliciting 50% response. Genotypes are arranged in order of least to most trichome coverage on the leaf.

	b (SE)	**c (SE)**	**d (SE)**	**e (SE)**
L. cul. CDC Redberry	1.415 (0.546)	0.021 (0.022)	0.291 (0.032)	802.407^{a} (208.078)
NAM 38-22	0.829 (0.36)	-0.012 (0.051)	0.388 (0.057)	534.716^{bc} (209.155)
NAM 38-55	1.862 (0.583)	0.015 (0.005)	0.16 (0.017)	573.818^{b} (135.142)
NAM 38-103	2.109 (0.665)	0.02 (0.004)	0.136 (0.014)	519.998^{cd} (115.856)
L. tom. IG 72805	0.745 (0.209)	0.002 (0.011)	0.231 (0.033)	115.946^{e} (52.145)
NAM 38-104	1.503 (0.615)	0.026 (0.009)	0.234 (0.03)	389.246^{f} (122.705)

Compact letter display shows significant differences between ED_{50} values after performing paired t-tests with alpha = 0.05.

4.4 Discussion

The first glyphosate dose-response study with *L. cul.* CDC Redberry and *L. tom.* IG 72805 suggested that *L. tom.* IG 72805 was more resistant to glyphosate when compared to *L. cul.* CDC Redberry due to having higher ED_{50} values when both injury and dry weight at 21 DAT were compared between the two (Tables 4.2 and 4.3). However, when these two genotypes were tested along with other NAM 38 sublines in the next dose-response experiment, the results were reversed. In the subsequent experiment, *L. cul.* CDC Redberry had a higher ED_{50} value than *L. tom.* IG 72805 for both injury and dry weight data, with the value being about 7 times higher after modelling dry weight 21 DAT (Tables 4.5 and 4.6). Not only were the trends reversed in the two experiments, but the ED_{50} values were also quite different. In the first experiment with only *L. cul.* CDC Redberry and *L. tom*. IG 72805, the ED_{50} values obtained after modelling dry weight data were 140 g ae/ha and 261 g ae/ha, respectively (Table 4.3). In the second experiment where *L. cul.* CDC Redberry and *L. tom.* IG 72805 were tested along with NAM 38 sublines, the ED_{50} values were 802 g ae/ha and 115 g ae/ha, respectively (Table 4.6). The plants in both iterations of the experiment were almost the same age upon treatment (plants in the first experiment were treated 21 DAS, and plants in the second experiment were treated 23 DAP after pre-germinating with ABA 1019 for 48 h). However, they were grown under slightly different temperatures and daylight regimes, and in different sized/shaped pots. The plants were sprayed in the spray chamber while they were in their respective pots as well, so some of the difference in results might be attributed to the difference in herbicide residue landing on individual plants while spraying. Another difference between the two experiments was that in the second experiment, seeds were pre-germinated using ABA 1019. Since extensive tests have not yet been done on how this ABA analog affects plant growth and development over time, it might be valuable to explore if herbicide metabolism is affected in plants treated with ABA 1019.

Trichome characteristics measured in these studies did not appear to affect the NAM 38 sublines response to glyphosate, as no clear trend in any of the parameter values was observed after modelling both injury and dry weight data and arranging the genotypes in the order of increasing trichome coverage (Table 4.6). However, when comparing ED_{50} values obtained after modelling the dry weight data, parents of the NAM 38 sublines, i.e., *L. cul.* CDC Redberry and *L. tom.* IG 72805 had the highest and lowest ED_{50} values, respectively, while the ED_{50} values of the rest of the NAM 38 sublines fell in between those of the parents (Table 4.6).

Based on the same data, *L. cul.* CDC Redberry appeared to be the most resistant to glyphosate and *L. tom*. IG 72805 seemed to be the most susceptible to glyphosate (Figure 4.10 and Table 4.6). A potential reason for this might be faster recovery in *L. cul.* CDC Redberry compared to *L. tom.* IG 72805 and other NAM 38 sublines. *L. cul*. CDC Redberry has increased biomass compared to *L. tom.* IG 72805, and thus, after treatment with glyphosate, it might have been able to grow rapidly and put on biomass more quickly in the case of intermediate doses which did not completely kill the plant. This could have led to increased dry weight in those plants, thus resulting in a higher overall ED_{50} value. However, the results for *L. cul*. CDC Redberry and *L. tom*. IG 72805 were not consistent with the ones obtained in the previous experiment, and more studies need to be conducted to ascertain the response of *L. cul*. CDC Redberry and *L. tom*. IG 72805 to varying doses of glyphosate.

The general trend for shoot spray retention remained mostly consistent for *L. cul.* CDC Redberry and *L. tom*. IG 72805 between the two experiments, even though plant ages were different, and they were grown under slightly different environmental conditions. In both experiments, after spraying *L. tom*. IG 72805 with water + surfactant, spray retention decreased compared to when it was sprayed only with water. While this difference was not statistically significant in the first experiment, in the second experiment when these two genotypes were tested along with other NAM 38 sublines, spray retention in the case of *L. tom.* IG 72805 was significantly lower after surfactant was added to the mix (Figure 4.5 and Table 4.4). These results suggest that the non-ionic surfactant Preference used in this experiment is ineffective at increasing retention in the case of *L. tom*. IG 72805. In the case of *L. cul*. CDC Redberry, while spray retention significantly increased after the addition of surfactant in the first experiment (Figure 4.5), in the second experiment, the difference in spray retention among the two treatments was inconsistent between the two units of measurements and was statistically insignificant (Table 4.4). The difference in statistical significance between the two experiments might be due to the age of the plants at the time of spraying – the plants in the second experiment were sprayed (along with other NAM 38 sublines) 24 DAP after pre-germinating them with ABA 1019 for 48 h, while in the first experiment they were sprayed 37 DAS. Similarly, the fact that the reduction in spray retention in *L. tom.* IG 72805 between the two treatments was not statistically significant in the first experiment (Figure 4.5) but was statistically significant in the second experiment (Table 4.4), suggests that the age of plants might influence spray retention in the presence of non-ionic surfactant.

Additionally, without surfactant, the baseline spray retention for *L. tom*. IG 72805 with just water was higher than that of *L. cul.* CDC Redberry when measured in µL/cm^2 for both experiments (Figure 4.5 and Table 4.4). This implies that spray retention is inherently higher in *L. tom*. IG 72805, meaning that *L. tom.* IG 72805 may have an increased ability to uptake foliar applied products when unmixed with non-ionic surfactant compared to *L. cul.* CDC Redberry, since higher retention has shown to increase herbicide efficacy (Peng et al., 2005). While this might not be beneficial for herbicides such as metribuzin that are used post-emergence in lentil as increased retention might result in increased absorbance and thus toxicity, it appears that the morphology of *L. tom.* IG 72805 might make it more responsive to the application of fungicides and insecticides.

From spray retention studies done on NAM 38 sublines, it is evident that as trichome density, length, and coverage increase, spray retention greatly decreases with the addition of the non-ionic surfactant (Figures 4.6, 4.7, and 4.8). These results suggest that lentil trichomes might prove to be a beneficial characteristic when using herbicides whose efficacy is increased when mixed with non-ionic surfactants. There are, however, other unexplored factors such as surface waxes and leaf angles that might affect droplet spread and retention in lentil, as shown in a variety of other plants (Holder, 2012; Manthey et al., 2009; Xu et al., 2011). For pre-harvest systemic herbicides used for desiccation such as glyphosate, it is beneficial to reduce herbicide residue in the seed to ensure that the level of glyphosate is below the MRL for the export market (Xu et al., 2019). In this context, increased trichomes on pods might reduce glyphosate retention and consequent absorption, thus limiting the amount of glyphosate translocated into the seed. Although there are a few non-ionic surfactants registered for use with glyphosate (Saskatchewan Ministry of Agriculture, 2020), glyphosate efficacy is not known to increase significantly when used with non-ionic surfactants (Norsworthy and Grey, 2004; Riechers et al., 1995). The use of non-ionic surfactants might then be limited in the case of glyphosate. Studies with other types of surfactants such as cationic and oil-based surfactants are thus warranted and may have a different effect on spray retention in lentil.

4.5 Conclusions

Glyphosate dose-response studies in the case of *L. cul.* CDC Redberry and *L. tom*. IG 72805 yielded inconsistent results, rendering these results inconclusive in terms of the degree of resistance/susceptibility of these genotypes to glyphosate. More studies are thus needed to

conclude how *L. cul.* CDC Redberry and *L. tom*. IG 72805 respond to varying doses of glyphosate. From the dose-response study done with NAM 38 sublines, it can be inferred that trichomes don't appear to be influencing glyphosate efficacy in lentil as no general trend with respect to ED_{50} values for dry weight or injury data was observed with increased trichome coverage.

Spray retention studies concluded that while adding surfactant in the case of *L. cul*. CDC Redberry led to increased retention, in the case of *L. tom*. IG 72805, retention decreased upon adding surfactant. Thus, subsequent pesticide absorption and efficacy may reduce in the case of *L. tom.* IG 72805 when the pesticide is mixed with non-ionic surfactant. While these results were consistent among both experiments, there were inconsistencies in the statistical significance of these results. This difference in statistical significance suggests that spray retention might be influenced by plant age, since plants in the two experiments varied in age by about two weeks and were grown in different greenhouses under slightly different environmental conditions. In both experiments, spray retention for *L. tom*. IG 72805 when sprayed only with water was higher compared to the spray retention for *L. cul*. CDC Redberry after spraying with water. This suggests that without surfactant, herbicide efficacy might be higher in *L. tom*. IG 72805 compared to *L. cul.* CDC Redberry.

For NAM 38 sublines, spraying with water + surfactant led to reduced retention as trichome density, length, and coverage increased, while spraying with only water had minimal effect on spray retention. These results suggest that it might be beneficial to breed for increased trichomes in lentil in order to reduce the efficacy of herbicides that are usually mixed with non-ionic surfactants. However, when planning to spray without the surfactant, increased trichomes might not be beneficial.

Chapter 5

Comparison of pea aphid fecundity and biosis on wild and cultivated lentil

5.1 Introduction and Objectives

Aphids are among the most economically important pests of agricultural crops in the world (Sorensen, 2009). Belonging to the order Hemiptera, they have piercing-sucking mouthparts and are specialized in feeding on the plant phloem, thereby draining the plants of their nutrients and causing them to wither and die (Smith and Chuang, 2014; Sorensen, 2009). Moreover, aphids serve as vectors for a range of devastating plant viruses such as the pea enation mosaic virus, potato leaf roll virus, broad bean wilt virus, etc. (Ng and Perry, 2004), making them especially egregious plant pests.

Although pea aphids (*Acyrthosiphon pisum* Harris) maintain a predominantly apterous (wingless) form during optimal conditions, with increase in environmental stress such as crowding, heightened risk of predation, or deteriorating host quality, females produce more winged (alate) offspring which enable their dispersion (Brisson, 2010; Reyes et al., 2019). In the Canadian prairies, pea aphids infest pea fields in the spring and as the peas start to mature, migrate onto lentil fields in the summer in early July/August. Some cultivated lentil genotypes were found to be moderately susceptible to pea aphid and were reported to exhibit antixenosis potential (non-preference by the insect) (Kordan et al., 2019) as well as antibiosis (negative influence on insect growth and survival) (Andarge, 2001). However, literature is lacking for wild lentil with respect to assessment of resistance to pea aphid.

This experiment aims to compare pea aphid fecundity and biosis in two cultivated lentil genotypes, CDC Maxim and CDC Redberry, and one wild lentil genotype, *L. tom.* IG 72805. *L. tom.* IG 72805 exhibits differences in morphological characteristics compared to *L. cul.* CDC Redberry and *L. cul.* CDC Maxim, such as extensive presence of trichomes on leaf and pod surface, prostrate growth habit, smaller leaflet size, and lower biomass. *L. cul.* CDC Redberry is a high-yielding small red lentil variety developed by the Crop Development Centre, University of Saskatchewan, Saskatoon, Canada in 2005 (Vandenberg et al., 2006). *L. cul.* CDC Maxim also belongs to the small red market class but is resistant to imidazolinone and is the most widely grown cultivar in Canada (McMurray et al., 2019). The objective of this

experiment is to determine if wild lentil, specifically *L. tom.* IG 72805, can serve as a potential genetic source of pea aphid resistance.

5.2 Materials and Methods

5.2.1 Plant material

Fifteen seeds each of *L. cul.* CDC Maxim, *L. cul.* CDC Redberry, and *L. tom.* IG 72805 were germinated by placing them in petri dishes using water treated with 10 µM of the abscisic acid analogue ABA 1019 (developed by Dr. Sue Abrams, patent in process) for 48 h. Germinated seeds were planted in 4-inch square pots filled with Sunshine Mix 4 (Sun Gro Horticulture, Canada), with 1 seed planted per pot. Pots were placed in a growth chamber in the phytotron facility at the University of Saskatchewan, Saskatoon, Canada. Plants were grown under the following environmental conditions: 16 h day at 21℃, 8 h darkness at 15℃, and at ambient humidity. Imprints of the adaxial leaf surface of *L. cul.* CDC Maxim were taken using SUMP discs and observed under a light microscope. Data for trichome density, trichome length, epidermal cell density, and stomatal index for the three genotypes are summarized in Table 5.1.

Table 5.1 Trichome density, trichome length, epidermal cell density, and stomatal index of the three genotypes used.

Genotype	No. of trichomes/mm^2	Trichome length (µm)	No. of epidermal cells/mm^2	Stomatal index
L. cul. CDC Redberry	2	332	650	20
L. cul. CDC Maxim	18	614	1167	18
L. tom. IG 72805	38	670	818	23

5.2.2 Maintenance of pea aphid colony

Pea aphids were obtained from a colony maintained at Agriculture and Agri-Food Canada, Saskatoon, Saskatchewan, which sourced aphids from pea fields in Saskatoon, Saskatchewan. Sequencing of the cytochrome *c* oxidase subunit 1 revealed that the pea aphid strain was 5A (Ningxing Zhou, personal communication). The experimental pea aphid colony was established and maintained on *L. cul.* CDC Maxim plants grown in a growth cabinet (Sanyo MLR-350H, Sanyo Electric Co. Ltd., Japan) at 20℃, 16:8 h light:dark cycle, and 70% RH in

the College of Agriculture and Bioresources building, University of Saskatchewan, Saskatoon, Canada (52°07'58.8"N, 106°37'51.6"W). Pea aphids were reared for at least eight generations before starting the experiment.

5.2.3 Aphid infestation and collection of data

Plants were infested with pea aphids during the pod development/flowering stage. This was at 75 DAP for *L. cul.* CDC Maxim and *L. tom.* IG 72805, and 98 DAP for *L. cul.* CDC Redberry. One apterous adult pea aphid in its last instar was released onto the tip of each plant. Tree Tanglefoot® Insect Barrier (Scotts Canada Ltd, Ontario, Canada) was applied on the branch after five nodes from the tip of the stem on which the aphid was placed. A sheer organza favour bag measuring 8 x 10 cm (Staruby, Amazon.ca) was placed and tied to the plant to enclose the aphid on the plant in the bag. The plants were then kept at 20℃, 16 h:8 h light:dark cycle, at 70% RH and pea aphid growth was monitored. Once the pea aphid had produced nymphs, all but one to four nymphs were removed from the plant. Time taken for these nymphs to reach reproductive stage was then tracked by recording the number of aphids on each plant every day. An aphid was considered to reach reproductive stage when it had produced at least one offspring or it had developed wings, at which point the plant was discarded. If the initial adult aphid placed on the plant did not produce nymphs, or if the nymphs died prematurely, then a new adult aphid was placed on a separate branch of the same plant and its nymphs tracked as previously described.

5.2.4 Statistical analysis

The following data were collected: Aphid mortality, i.e., the number of aphids (nymphs as well as adults) that died on the plant; Maturity time, i.e. the days taken for the nymph to reach reproductive stage; Maximum adult size, i.e., the size (in mm), of the biggest adult on the plant after reaching maturity; and Number of winged aphids, i.e., the number of aphids (both nymphs and adults) which developed wings on the plant. All analyses were performed using R Statistical Software version 3.6.0 (R Core Team, 2019). Differences in aphid mortality were examined between the three genotypes separately at the nymph and adult stages (i.e., six categories in total) using Kruskal-Wallis rank-sum test (R package agricolae; de Mendiburu, 2019) and post hoc testing was conducted using Dunn's test of multiple comparison (R package FSA; Ogle et al., 2020) with p-values adjusted via Bonferroni method using alpha = 0.05. Differences in maturity time and number of winged aphids between each genotype (i.e., between three categories in total) were also examined using Kruskal-Wallis rank-sum test. Post

hoc testing was done using Dunn's test of multiple comparison with alpha = 0.05 with p-values adjusted via Bonferroni method. Maximum adult size achieved on each genotype was examined using GLM with genotype as the fixed factor and adult size as the response. This model was chosen after comparing dispersion and Akaike information criteria (AIC) values between GLMs with a Poisson or negative binomial probability distribution. Post hoc testing was conducted using Tukey's method of comparing least-squares means using emmeans package in R (Lenth, 2019). Other packages used in the analyses were car (Fox and Weisberg, 2019), rcompanion (Mangiafico, 2019), dunn.test (Dinno, 2017), multcomp (Hothorn et al., 2008), nlme (Pinheiro et al., 2019), and pgirmess (Giraudoux, 2018). All figures were made using the R Package ggplot2 (Wickham, 2016).

5.3 Results

While majority of the plants of all three genotypes remained healthy until the end of the experiment, three plants from the genotype *L. cul.* CDC Redberry and two plants from the genotypes *L. cul.* CDC Maxim and *L. tom.* IG 72805 wilted before the experiment could be completed and were thus removed from analyses. There were also instances of aphids getting stuck on Tanglefoot® or escaping from organza bags, which required new adult aphids to be placed on new branches so that their nymphs could be tracked until maturity. Aphids that died due to sticking to Tanglefoot® were not included in these analyses.

Total aphid mortality was highest for *L. cul.* CDC Maxim, on which mean combined adult and nymph deaths were approximately seven per plant, while on *L. cul.* CDC Redberry and *L. tom.* IG 72805, the mean mortality was about six and one per plant, respectively (Figure 5.1). The effects of genotype (Kruskal-Wallis: $\chi^2 = 19.61$, df = 2, p = <0.0001), growth stage (Kruskal-Wallis: $\chi^2 = 8.56$, df = 1, t-value = 1.99, p = 0.003), and the interaction between genotype and growth stage (Kruskal-Wallis: $\chi^2 = 28.30$, df = 5, p = <0.0001) were all significant on aphid mortality. Post hoc testing revealed that both adult and nymph mortality was significantly higher on *L. cul.* CDC Maxim compared to respective adult and nymph mortalities on *L. tom.* IG 72805 (Figure 5.1). Adult mortality on *L. tom.* IG 72805 was also significantly lower than nymph mortality on *L. cul.* CDC Redberry and *L. cul.* CDC Maxim (Figure 5.1). Complete results of post hoc testing with test-statistics and p-values can be found in Appendix 17.

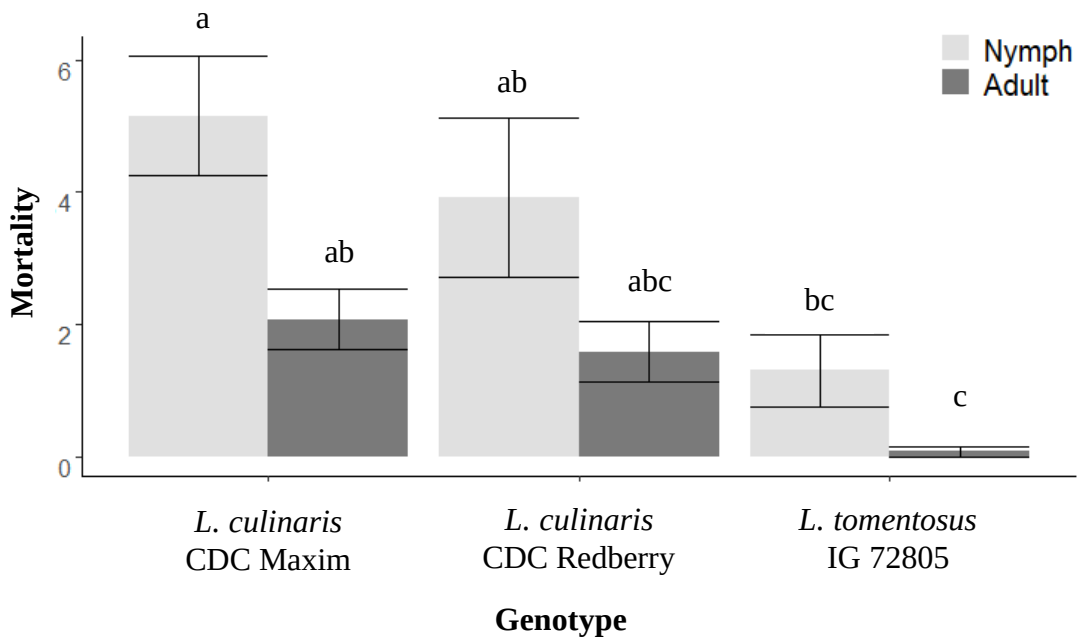


Figure 5.1 Mortality of aphid nymphs and adults on three lentil genotypes under controlled conditions. Error bars are standard error of means and compact letter displays show significant difference at p = 0.05.

Maturity time also differed significantly between genotypes (Kruskal-Wallis: χ^2 = 15.136, df = 2, p = 0.0005). The mean number of days to maturity on *L. cul.* CDC Maxim was approximately 16, while on *L. cul.* CDC Redberry and *L. tom.* IG 72805 was 10 and 11 d, respectively. Even though the pea aphid colonies were maintained on *L. cul.* CDC Maxim, the time required for nymphs to reach maturity was observed to be the longest for the cultivar CDC Maxim. Post hoc testing using Dunn test revealed that this was significantly longer than the time required for nymphs to reach maturity on *L. cul.* CDC Redberry and *L. tom.* IG 72805 (Figure 5.2 A). Appendix 18 contains Z-values and p-values for pairwise comparisons for difference in maturity time in three genotypes after adjusting p-values using Bonferroni method.

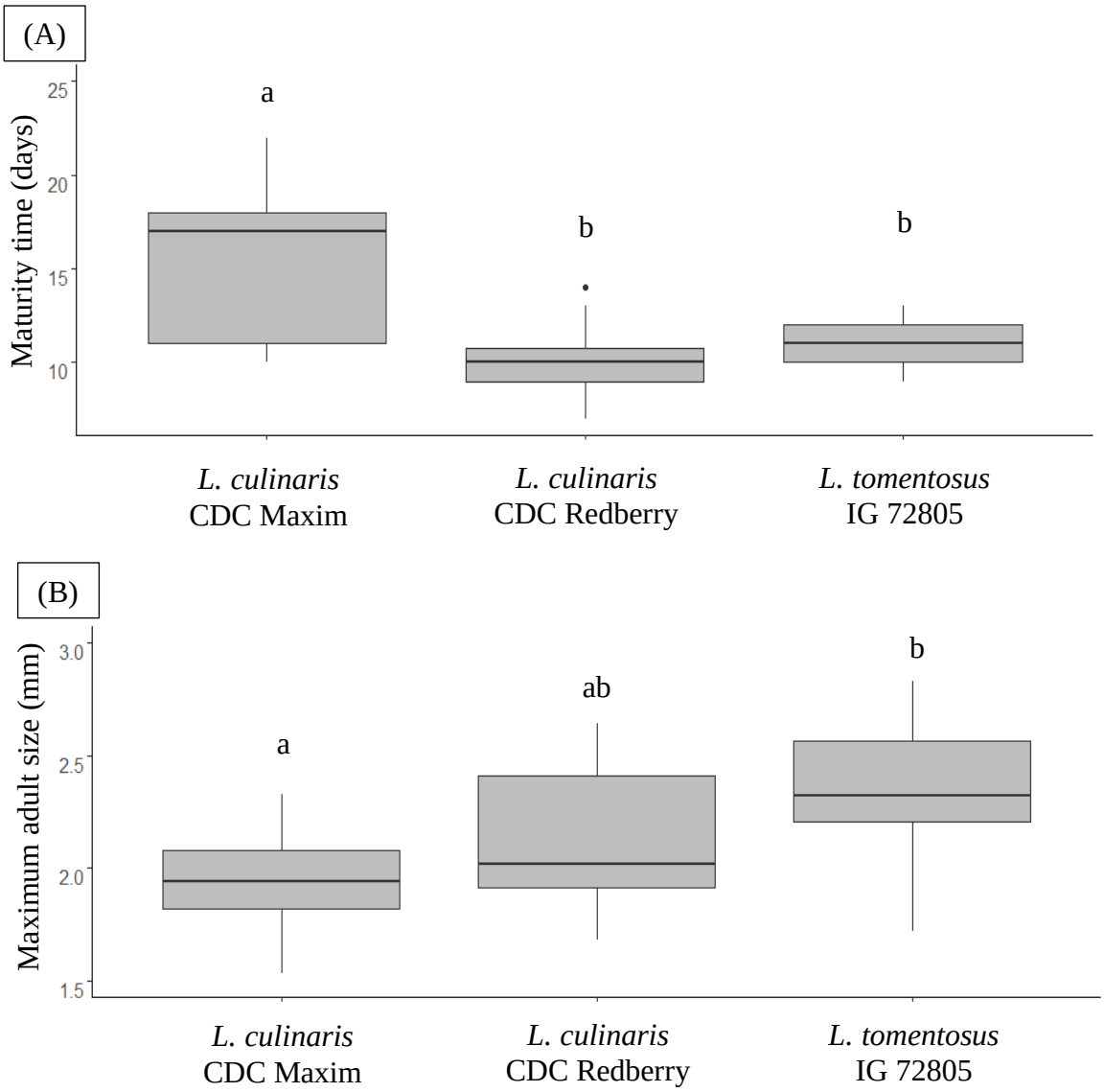


Figure 5.2 (A) Maturity time and (B) Maximum size reached by adult pea aphids while feeding on *L. cul.* CDC Maxim (commercial control), *L. cul.* CDC Redberry, and *L. tom.* IG 72805 under controlled conditions. Compact letter displays show significant difference at p = 0.05.

Maximum adult size after the nymphs had attained reproductive maturity varied significantly between genotypes (F = 6.4553, df = 2, p = 0.004) (Figure 5.2 B, Table 5.2). While feeding on *L. tom.* IG 72805, adult aphids were, on average, 2.4 mm long, while aphids measured an average of 1.9 mm and 2.1 mm after feeding on *L. cul.* CDC Maxim and *L. cul.* CDC Redberry, respectively. Post hoc testing revealed that pea aphids grew significantly larger on *L. tom.* IG 72805 compared to *L. cul.* CDC Maxim, but there was no significant difference between the size of aphids reared on *L. cul.* CDC Redberry compared to the other genotypes (Figure 5.2 B). Results after comparing least-squares means between pairs are reported in Appendix 19.

Table 5.2 Results for maximum adult size of pea aphids after fitting GLM.

Source	t-value	p (>|t|)
L. cul. CDC Maxim	23.949	< 0.0001
L. cul. CDC Redberry	1.554	0.1299
L. tom. IG 72805	3.592	0.0011

Residual deviance = 2.74, residual df = 32, dispersion parameter = 0.08.

The number of aphids that developed wings did not differ significantly among the three genotypes (Kruskal-Wallis: χ^2 = 2.5953, df = 2, p = 0.273). Most of the plants of all three genotypes had zero instances of aphids developing wings (Figure 5.3). Mean number of aphids per plant that developed wings were 1.15 and 1.17 for *L. cul.* CDC Maxim and *L. cul.* CDC Redberry respectively, and 0.23 for *L. tom.* IG 72805.

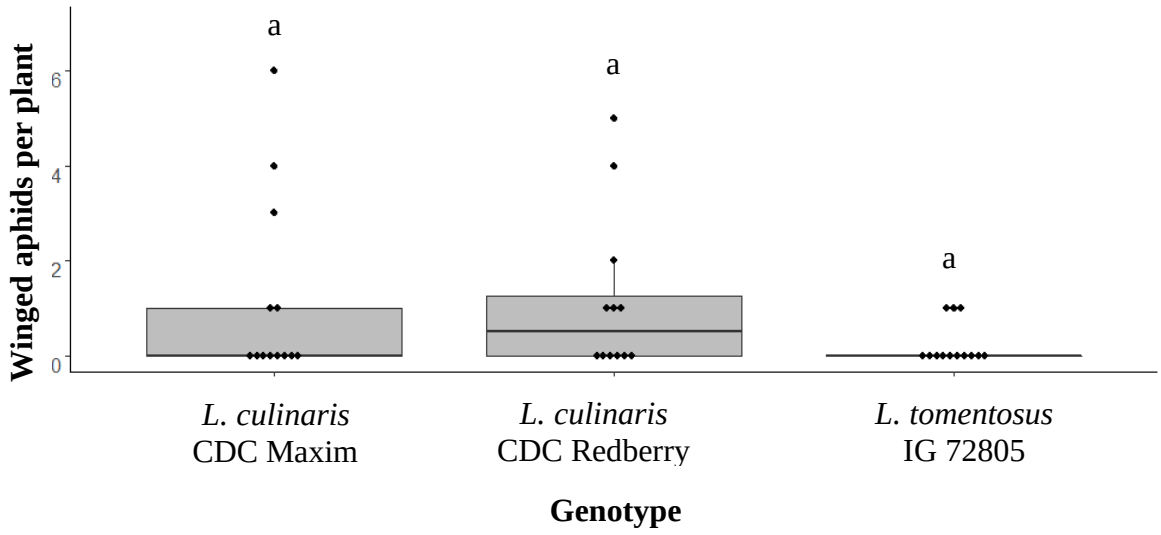


Figure 5.3 Total winged aphids per plant on *L. cul.* CDC Maxim (commercial control), *L. cul.* CDC Redberry, and *L. tom.* IG 72805 under controlled conditions. Compact letter displays show significant difference at p = 0.05.

5.4 Discussion

Both adult and nymph mortality was lowest in *L. tom.* IG 72805 compared to *L. cul.* CDC Maxim and *L. cul.* CDC Redberry, indicating that *L. tom.* IG 72805 served as the best host for pea aphid (Figure 5.1). Mortality on *L. cul.* CDC Redberry was not significantly different from

either *L. tom.* IG 72805 or *L. cul.* CDC Maxim, indicating that *L. cul.* CDC Redberry did not exhibit any significant antibiosis effects that were absent from *L. cul.* CDC Maxim or *L. tom.* IG 72805. Rearing on *L. cul.* CDC Maxim, however, led to significantly higher mortality compared to *L. tom.* IG 72805, in the case of both adults and nymphs. Several factors may contribute to this observed phenomenon of partial resistance to the pea aphid observed in *L. cul.* CDC Maxim.

Pea aphids primarily depend on the phloem sap to fulfill their nutritional requirements. Even though the sap contains adequate sugars and most other nutritive components, it is typically lacking in amino acids essential for aphid growth and reproduction (Douglas, 1993). This lack of free amino acids in the phloem sap has been linked to contributing to aphid resistance (Auclair, 1976; Febvay et al., 1988). To fulfill their amino acid requirements, aphids associate obligatorily and symbiotically with the bacteria *Buchnera* that synthesize amino acids lacking in the aphids' diet, and without *Buchnera*, aphid development and fecundity are severely negatively affected (Douglas, 1998). Although feeding was not measured in this experiment, it may be that the phloem sap of *L. cul.* CDC Maxim lacks specific amino acids or nutritive compounds necessary for the survival of pea aphid or *Buchnera*.

Other factors contributing to aphid resistance include efficiency of phytohormonal signalling via salicylic acid and jasmonic acid eliciting a chemical defence response through plant secondary metabolites, or a physical response of sealing of sieve tube via callose deposition or protein plugging as a result of stylet penetration (Will et al., 2013; Züst and Agrawal, 2016). One or more of these responses might be more effective in *L. cul.* CDC Maxim, and *L. tom.* IG 72805 might be unable to respond to biotic stress damage as efficiently as *L. cul.* CDC Maxim. Extensive studies are needed to explore each of these aspects and understand physiological differences in response to aphid infestation among wild and cultivated lentil genotypes.

The time taken for nymphs to reach reproductive stage was highest in *L. cul.* CDC Maxim (approximately 16 d) which significantly differed from *L. cul.* CDC Redberry and *L. tom.* IG 72805 (about 10 d each) (Figure 5.2 (A)). This result was unexpected, especially for *L. cul.* CDC Maxim, since prior to infestation of experimental plants, aphids were taken from the stock colony which was maintained on *L. cul.* CDC Maxim. It was expected that the aphids would be accustomed to being reared on *L. cul.* CDC Maxim and would mature faster when compared to *L. cul.* CDC Redberry and *L. tom.* IG 72805, both of which were new genotypes for these

aphids. Maturity time on *L. cul.* CDC Redberry was significantly lower than that on *L. cul.* CDC Maxim, indicating that there might be intraspecific differences within *L. culinaris* as it relates to phloem nutrients. Considering how aphid mortality was similar on *L. cul.* CDC Maxim and *L. cul.* CDC Redberry, it appears that once aphids overcome physical and chemical barriers posed by the two species, they rapidly grow on *L. cul.* CDC Redberry while they grow significantly slower on *L. cul.* CDC Maxim. Perhaps *L. cul.* CDC Maxim is lacking in micro and macronutrients essential for aphid growth and development, while *L. cul.* CDC Redberry and *L. tom.* IG 72805 have more of these nutrients that facilitate rapid aphid growth. Studies can be conducted to explore the nutritional differences between these three genotypes. *L. cul.* CDC Redberry and *L. cul.* CDC Maxim also have tougher stems which have been selected for upright growth habit and reduced lodging. *L. tom.* IG 72805, on the other hand, has a more prostrate growth habit. It is possible that nymphs and adults had difficulty inserting their stylets into the stem tissue to access the phloem sap of *L. cul.* CDC Maxim and *L. cul.* CDC Redberry, but were easily able to pierce through the softer stem tissue of *L. tom.* IG 72805. This can be tested by conducting feeding studies using electronic monitoring systems that can distinguish between various stages of feeding including salivation, penetration of the plant tissue, and sap sucking or ingestion (Brown and Holbrook, 1976). Another obvious difference in morphology of *L. culinaris* and *L. tomentosus* is the extensive presence of trichomes on *L. tomentosus.* In this experiment, trichomes present on *L. tom.* IG 72805 did not seem to confer any advantage in terms of providing aphid resistance. This is similar to the conclusion reached upon by Gao et al. (2008) after examining the role of non-glandular trichomes of *Medicago truncatula* (Gao et al., 2008).

Mature aphids feeding on *L. tom.* IG 72805 attained the largest size, while aphids feeding on *L. cul.* CDC Maxim were the smallest (Figure 5.2 (B)). On *L. cul.* CDC Redberry, aphids reached a size in between that of *L. cul.* CDC Maxim and *L. tom.* IG 72805. These results agree with the general trend observed thus far: pea aphids seem to have increased fecundity and biosis on *L. tom.* IG 72805, while *L. cul.* CDC Maxim seems to be the host of lowest quality. In addition to possible reasons for reduced growth and development on *L. culinaris* mentioned previously, another factor that could influence adult size on *L. cul.* CDC Maxim and *L. cul.* CDC Redberry might be that, on these genotypes, more aphids developed wings as compared to *L. tom.* IG 72805 (Figure 5.3). It is well documented that alate aphids are smaller than apterous individuals (Brisson, 2010; Zhang et al., 2016b). Apterous aphids are larger, and this

leads to increased nymph production, while alate aphids have reduced size and fertility and their physiology is primarily suited for flight dispersal (Braendle et al., 2006).

There was no statistically significant difference among number of winged adults on the three genotypes. This was likely because the female aphids that were initially put on each plant came from the same stock colony, and it is the parthenogenic female that produces a high proportion of winged morphs after sensing environmental stress and then transferring environmental cues to developing embryos (Braendle et al., 2006; Brisson, 2010; Ogawa and Miura, 2014). It may be that in the stock colony, the density of aphids was increasing, which led to increased production of winged morphs on our experimental plants. Moreover, it was observed that a lot of aphids in the colony were developing wings around late July-August while this experiment was ongoing. This was also when winged aphids were observed in lentil fields outdoors (Personal Observation). The increase in alate morphs might be seasonal and/or temporal, as has also been observed in other aphid species (Dixon, 1985; Mehrparvar et al., 2013).

5.5 Conclusions

This experiment established that *L. tom.* IG 72805 was the best host for pea aphid when compared to *L. cul.* CDC Maxim and *L. cul.* CDC Redberry. *L. tom.* IG 72805 had the lowest aphid mortality for both adults and nymphs, largest aphid size, and aphids took the least time to mature on *L. tom.* IG 72805. *L. cul.* CDC Maxim showed the best antibiosis potential against pea aphid since aphid mortality was highest on *L. cul.* CDC Maxim for adults and nymphs alike, and aphids took the longest time to mature while attaining the smallest size. Although aphid growth and development were retarded in *L. cul.* CDC Redberry compared to *L. tom.* IG 72805, for most of the parameters measured, *L. cul.* CDC Redberry did not differ significantly from the other two genotypes. These results indicate that the high trichome density on leaves and pods of *L. tom.* IG 72805 did not negatively affect the performance of the pea aphid.

There are many possible reasons as to why aphid fecundity and biosis was substantially increased in *L. tom.* IG 72805 but was reduced in *L. cul.* CDC Redberry and was significantly lower in *L. cul.* CDC Maxim. These factors include differences in phloem content such as amino acids and sugars, and chemicals involved in plant defense such as phenolic compounds and alkaloids. Moreover, physical characteristics of *L. culinaris* are different from that of *L. tomentosus*, such as more lignified stem tissue, and this may play a role in aphid deterrence.

While a deeper look into all these possibilities is warranted to identify resistance mechanisms, it appears that during the course of selecting varieties for commercial production, especially in the case of selection for improvement in lodging tolerance (stiffer stems) there may have been indirect selection for mild aphid resistance in lentil cultivars.

Chapter 6
General discussion and future work

The issues of drought, herbicide susceptibility, and insect pressure exacerbated by climate change pose inevitable threat to future lentil production in the Canadian prairies. It is thus important to breed cultivars that can withstand these stresses so as to sustain lentil production in Canada. Efforts are underway to use wild relatives of lentil as genetic resources in identifying resistant traits to develop new and improved cultivars through intercrossing (Gupta and Sharma, 2006; Singh et al., 2013; Tullu et al., 2011). This project focused on the trait of pubescence and its influence in drought tolerance, herbicide resistance, and insect resistance in lentil. The primary goal of this project was to determine if trichomes are a valuable trait to breed into cultivated lentil.

Genotypes of cultivated and wild lentil species were grown under moderate drought (40% FC) and FW conditions and weight loss due to transpiration was determined. Rate of transpiration differed between species, with genotypes of *L. tom.* and *L. ode.* grown under FW condition having higher rate of transpiration compared to others. Most genotypes had adapted to lack of soil moisture and had a reduced rate of transpiration when grown under 40% FC compared to their counterparts grown under FW condition, but *L. cul.* CDC Redberry and *L. tom.* IG 72613 had a slightly increased rate of transpiration under 40% FC condition. While these differences may be attributed to lack of replication since they were not statistically significant, if real, they show existing variability in both *L. cul.* and *L. tom.* genotypes. A great deal of variability was also observed between species as well as genotypes within the same species after examining the parameter of trichome density, and some variability was observed in trichome length, epidermal cell density, and stomatal length. Genotype *L. erv.* IG 72815 had lowest trichome density and length under both FW and 40% FC conditions and genotypes *L. tom.* IG 72613 and *L. ode.* IG 72623 had highest trichome density of all genotypes when grown under FW and 40% FC conditions, respectively. Out of the four cultivars tested in this experiment, only *L. cul.* CDC Greenstar increased both its trichome density and epidermal cell density in response to drought. Due to inconsistency in regulation of trichome density and the regulation of transpiration rate, a clear connection between trichome characteristics could not be established in relation to drought avoidance or tolerance. However, trichome density did seem to be associated with transpiration rate since most genotypes that increased their trichome density under moderate drought also had reduced transpiration rate, and vice versa. Overall, results

from this experiment suggest that genotypes within the same species regulate their anatomy and physiology in different ways in response to drought and thus need to be considered independently instead of assuming the same behaviour for all genotypes within one species. Although this variability makes it difficult to predict the behaviour of genotypes via their species, it serves to strengthen the genetic base in our breeding program and can be further exploited to develop improved lentil varieties.

The influence of trichomes on herbicide tolerance and spray retention was studied via experiments done on F_5-F_7 derived lines from interspecific NAM 38 (*L. cul.* CDC Redberry x *L. tom.* IG 72805) population. Lines in this population were segregating for trichome characteristics and were chosen to represent a wide range in trichome density and length on the adaxial leaf surface. Results from glyphosate tolerance studies were inconsistent and no correlation was found between trichome density, length, or coverage, and glyphosate tolerance. However, water spray retention was found to decrease with increasing trichome density, length, and coverage when non-ionic surfactant was mixed in the spray solution. This shows that enhanced trichome coverage might be beneficial in increasing lentil resistance to foliar-applied products that require non-ionic surfactants as adjuvants. These results might have a key role to play considering the issue of glyphosate residue in lentil seeds which has already resulted in trade restrictions by the EU in the past (Pratt, 2011). Reduced herbicide retention due to increased trichomes on pods might result in less subsequent absorption of glyphosate into the seeds when glyphosate is sprayed pre-harvest for desiccation. However, the reduction in retention in this experiment was observed when water was mixed with 0.25% v/v non-ionic surfactant and thus specific studies need to be conducted to determine if a similar effect is observed with glyphosate. Moreover, retention differs based on the choice of surfactant used (Harbour et al., 2003; Nalewaja et al., 1996; Reddy et al., 1995) and further studies using surfactants specific to glyphosate or other desiccants need to be done to ascertain if trichomes continue to serve as a barrier.

While the results from spray retention study can be extrapolated to other foliar applied products such as disease- and pest-management products (fungicides and insecticides), more specialized studies are still needed to optimize spray parameters such as nozzle orientation and droplet size. The uniformity of spray coverage may also be a factor, not just at the top of the plant canopy, but also in the middle and the bottom of the plant canopy where diseases and insects are more likely to manifest (Bruns and Nalewaja, 1999; Gossen et al., 2008). Moreover, the

influence of various other factors that influence foliar spray retention cannot be ruled out, for example, leaf angle, orientation, and arrangement on the canopy (Ennis et al., 1952; Massinon et al., 2014).

Sublines of the interspecific NAM 38 population (*L. cul.* Redberry x *L. tom.* IG 72805) at F_5-F_7 generation used in this experiment are currently available in the breeding program at the University of Saskatchewan and can be further tested in the field for a more in-depth performance evaluation. These lines have varying trichome density, length, and coverage among other leaf and canopy characteristics and may serve useful to determine beneficial agronomic traits from wild lentil *L. tom.* IG 72805.

Experiments with pea aphid suggested that trichomes on *L. tom.* IG 72805 do not impede aphid growth and development, as aphids matured faster and grew bigger on *L. tom.* IG 72805 compared to *L. cul.* CDC Maxim and *L. cul.* CDC Redberry. However, these results might be due to reasons other than trichomes, since *L. tom.* and *L. cul.* differ in multiple traits such as leaflet size, plant biomass, and growth habit (Singh et al., 2014), as well as unexplored factors such as nutrition content in the phloem and stem lignin content. Cultivars used in this study were selected over years of field trials for increased yield and resistance to lodging, but the actual mechanisms causing deterioration of aphid growth parameters remain unknown. Further studies can be conducted to identify genes and mechanisms associated with reduced aphid biosis on *L. cul.* CDC Maxim to develop aphid resistant cultivars.

An important consideration for the results obtained in this project is that conclusions are based on trichome density only on the adaxial surface of the leaflets. For herbicide spray retention studies this might be enough, since the adaxial surface primarily intercepts herbicide spray droplets, and its cuticle thickness and low stomatal density serve as main potential barriers to herbicide entry (Chachalis et al., 2001; Procópio et al., 2003). However, to obtain conclusive results related to drought tolerance and insect resistance, trichomes on the abaxial leaflet surface as well as pubescence on all aerial surfaces such as stems, pods, etc. should be examined. Similarly, studying the properties of the cuticle and the composition of epicuticular wax layer in wild and cultivated lentil species might prove useful in addressing the issues of drought tolerance, herbicide resistance, and insect resistance.

Last but not least, the interplay between biotic and abiotic stresses mentioned in this project could be further studied to better determine the effects of climate change on lentil production. Future studies could focus on how aphid biosis is affected in wild and cultivated lentil species grown under FW and 40% FC condition, and if plants of any genotype are resistant to insects under drought. Studies in pea and soybean have shown that aphid populations declined significantly in plants grown under drought vs. watered plants, possibly due to altered phloem sap viscosity in drought-stressed plants resulting in reduced feeding (Guo et al., 2015; Mcvean and Dixon, 2001; Nachappa et al., 2016). However, no such studies have been done in lentil. Similarly, the effect of drought stress on spray retention and glyphosate tolerance would also be appropriate to explore in lentil. Drought stress is known to reduce herbicide efficacy and to cause weeds to be more tolerant to herbicides (Devine et al., 1995; Xie et al., 1997; Zhou et al., 2007). Given the morphological changes in lentil observed under drought, and the decreased retention of water spray droplets upon the addition of the non-ionic surfactant, it would be pertinent to further explore changes in spray retention and herbicide tolerance among different lentil species and cultivars under drought conditions.

References

Abdulraham, A. A., and Oladele, F. A. (2011). Response of trichomes to water stress in two species of *Jatropha*. *Insight Bot.* 1, 15–21. doi:10.5567/botany-ik.2011.15.21.

Agrawal, A. A., Fishbein, M., Jetter, R., Salminen, J.-P., Goldstein, J. B., Freitag, A. E., et al. (2009). Phylogenetic ecology of leaf surface traits in the milkweeds (*Asclepias* spp.): chemistry, ecophysiology, and insect behavior. *New Phytol.* 183, 848–867. doi:10.1111/j.1469-8137.2009.02897.x.

Agriculture and Agri-Food Canada (2019). Canada: Outlook for principal field crops, 2019-07-19.

Al Charideh, A. R., and Abou Zakhem, B. (2010). Distribution of tritium and stable isotopes in precipitation in Syria. *Hydrol. Sci. J.* 55, 832–843. doi:10.1080/02626667.2010.487977.

Andarge, A. (2001). Aspects of bio-intensive pea aphid, *Acyrthospihon pisum* (Harris) management on lentil, *Lens culinaris* (Medikus). Available at: http://scholar.ufs.ac.za:8080/xmlui/handle/11660/6287 [Accessed September 24, 2019].

Aston, M. J., and Jones, M. M. (1976). A study of the transpiration surfaces of *Avena sterilis* L. var. Algerian leaves using monosilicic acid as a tracer for water movement. *Planta (Bed.)* 130, 121–129.

Auclair, J. L. (1976). "Feeding and nutrition of the pea aphid, *Acyrthosiphon Pisum* (Harris), with special reference to amino acids," in *The Host-Plant in Relation to Insect Behaviour and Reproduction*, ed. T. Jermy (Boston, MA: Springer US), 29–34. doi:10.1007/978-1-4613-4274-8_3.

Avé, D. A., Gregory, P., and Tingey, W. M. (1987). Aphid repellent sesquiterpenes in glandular trichomes of *Solanum berthaultii* and *S. tuberosum*. *Entomol. Exp. Appl.* 44, 131–138. doi:10.1111/j.1570-7458.1987.tb01057.x.

Aydin, A. L. P., Yesilmen, S., Vural, A., and Guran, H. S. (2010). Determination of some agronomical characteristics and Ochratoxin-A level of Karacadag rice (*Oryza sativa* L.) in Diyarbakir ecological conditions, Turkey. *African J. Agric. Res.* 5, 1965–1972.

Aydin, M. C., and Işhik, E. (2015). Evaluation of ground snow loads in the micro-climate regions. *Russ. Meteorol. Hydrol.* 40, 741–748. doi:10.3103/S1068373915110047.

Baldocchi, D. D., Verma, S. B., Rosenberg, N. J., Blad, B. L., Garay, A., and Specht, J. E. (1983). Leaf pubescence effects on the mass and energy exchange between soybean canopies and the atmosphere. *Agron. J.* 75, 537. doi:10.2134/agronj1983.00021962007500030028x.

Bates, D., Mächler, M., Bolker, B., and Walker, S. (2015). Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* 67, 1–48. doi:10.18637/jss.v067.i01.

Beckie, H. J., Gulden, R. H., Shaikh, N., Johnson, E. N., Willenborg, C. J., Brenzil, C. A., et al. (2015). Glyphosate-resistant kochia (*Kochia scoparia* L. Schrad.) in Saskatchewan and Manitoba. *Can. J. Plant Sci.* 95, 345–349. doi:10.4141/cjps-2014-328.

Benzing, D. H., and Burt, K. M. (1970). Foliar permeability among twenty species of the Bromeliaceae. *Bull. Torrey Bot. Club* 97, 269–279. doi:10.2307/2483646.

Beranová, R., and Kyselý, J. (2018). Trends of precipitation characteristics in the Czech Republic over 1961-2012, their spatial patterns and links to temperature and the North

Atlantic Oscillation. *Int. J. Climatol.* 38, e596–e606. doi:10.1002/joc.5392.

Bosabalidis, A. M., and Kofidis, G. (2002). Comparative effects of drought stress on leaf anatomy of two olive cultivars. *Plant Sci.* 163, 375–379. doi:10.1016/S0168-9452(02)00135-8.

Boutin, C., Aya, K. L., Carpenter, D., Thomas, P. J., and Rowland, O. (2012). Phytotoxicity testing for herbicide regulation: Shortcomings in relation to biodiversity and ecosystem services in agrarian systems. *Sci. Total Environ.* 415, 79–92. doi:https://doi.org/10.1016/j.scitotenv.2011.04.046.

Braendle, C., Davis, G. K., Brisson, J. A., and Stern, D. L. (2006). Wing dimorphism in aphids. *Heredity (Edinb).* 97, 192–199. doi:10.1038/sj.hdy.6800863.

Brand, J., Yaduraju, N. T., Shivakumar, B. G., and Murray, L. (2007). "Weed management," in *Lentil*, eds. S. S. Yadav, D. L. McNeil, and P. C. Stevenson (Dordrecht: Springer Netherlands), 159–172. doi:10.1007/978-1-4020-6313-8_10.

Brewer, C. A., and Smith, W. K. (1997). Patterns of leaf surface wetness for montane and subalpine plants. *Plant, Cell Environ.* 20, 1–11. doi:10.1046/j.1365-3040.1997.d01-15.x.

Brisson, J. A. (2010). Aphid wing dimorphisms: linking environmental and genetic control of trait variation. *Philos. Trans. R. Soc. B Biol. Sci.* 365, 605–616. doi:10.1098/rstb.2009.0255.

Brisson, J. A., and Davis, G. K. (2008). "Pea aphid," in *Genome Mapping and Genomics in Arthropods* (Berlin, Heidelberg: Springer Berlin Heidelberg), 59–67. doi:10.1007/978-3-540-73833-6_5.

Brisson, J. A., and Stern, D. L. (2006). The pea aphid, *Acyrthosiphon pisum*: an emerging genomic model system for ecological, developmental and evolutionary studies. *BioEssays* 28, 747–755. doi:10.1002/bies.20436.

Brown, C. M., and Holbrook, F. R. (1976). An improved electronic system for monitoring feeding of aphids. *Am. Potato J.* 53, 457–462. doi:10.1007/BF02852659.

Bruinsma, J. (2009). The resource outlook to 2050: By how much do land, water, and crop yields need to increase by 2050? in *Expert Meeting on How to Feed the World in 2050* (Food and Agriculture Organization of the United Nations, Economic and Social Development Department). Available at: ftp://ftp.fao.org/agl/aglw/docs/ResourceOutlookto2050.pdf [Accessed September 27, 2017].

Bruns, D. E., and Nalewaja, J. D. (1999). Spray retention is affected by spray parameters, species, and adjuvants. *ASTM Spec. Tech. Publ.*, 107–119. doi:10.1520/stp14158s.

Burrows, G. E., White, R. G., Harper, J. D. I., Heady, R. D., Stanton, R. A., Zhu, X., et al. (2013). Intrusive trichome bases in the leaves of silverleaf nightshade (*Solanum elaeagnifolium*; Solanaceae) do not facilitate fluorescent tracer uptake. *Am. J. Bot.* 100, 2307–2317. doi:10.3732/ajb.1300034.

Carlisle, L. (2016). Making heritage: The case of black Beluga agriculture on the Northern Great Plains. *Ann. Am. Assoc. Geogr.* 106, 130–144. doi:10.1080/00045608.2015.1086629.

Cessna, A. J., Darwent, A. L., Townley-Smith, L., Harker, K. N., and Kirkland, K. J. (2000). Residues of glyphosate and its metabolite AMPA in canola seed following preharvest applications. *Can. J. Plant Sci.* 80, 425–431. doi:10.4141/P99-062.

Chachalis, D., Reddy, K. N., Elmore, C. D., and Steele, M. L. (2001). Herbicide efficacy, leaf structure, and spray droplet contact angle among *Ipomoea* species and smallflower morningglory. *Weed Sci.* 49, 628–634. Available at: http://www.jstor.org/stable/4046461.

Dahlin, R. M., Brick, M. A., and Ogg, J. B. (1992). Characterization and density of trichomes on three common bean cultivars. *Econ. Bot.* 46, 299–304. doi:10.2307/4255445.

Davies, P. A., Lülsdorf, M. M., and Ahmad, M. (2007). "Wild relatives and biotechnological approaches," in *Lentil*, eds. S. S. Yadav, D. L. McNeil, and P. C. Stevenson (Dordrecht: Springer Netherlands), 225–240. doi:10.1007/978-1-4020-6313-8_14.

Davis, T. S., Wu, Y., and Eigenbrode, S. D. (2015). Chickpea variety and phenology affect acquisition of *Pea enation mosaic virus* , subsequent plant injury and aphid vector performance. *Ann. Appl. Biol.* 167, 420–425. doi:10.1111/aab.12239.

de Mendiburu, F. (2019). agricolae: Statistical procedures for agricultural research. Available at: https://cran.r-project.org/package=agricolae.

Délye, C., Jasieniuk, M., and Le Corre, V. (2013). Deciphering the evolution of herbicide resistance in weeds. *Trends Genet.* 29, 649–658. doi:https://doi.org/10.1016/j.tig.2013.06.001.

Deniz, A., Toros, H., and Incecik, S. (2011). Spatial variations of climate indices in Turkey. *Int. J. Climatol.* 31, 394–403. doi:10.1002/joc.2081.

Devine, M. D., Harren, J. C., Abrams, S. R., and Gusta, L. V. (1995). Effect of drought stress, abscisic acid, and abscisic acid analogues on the efficacy of diclofop-methyl and tralkoxydim. *J. Plant Growth Regul.* 14, 77–84. doi:10.1007/BF00203117.

Dinno, A. (2017). dunn.test: Dunn's test of multiple comparisons using rank sums. Available at: https://cran.r-project.org/package=dunn.test.

Dixon, A. F. G. (1985). "Resource tracking: mechanism — polyphenism," in *Aphid ecology: An optimization approach* (New York, NY: Springer Netherlands), 110. doi:10.1007/978-94-011-5868-8.

Douglas, A. E. (1993). The nutritional quality of phloem sap utilized by natural aphid populations. *Ecol. Entomol.* 18, 31–38. doi:10.1111/j.1365-2311.1993.tb01076.x.

Douglas, A. E. (1998). Nutritional interactions in insect-microbial symbioses: aphids and their symbiotic bacteria *Buchnera*. *Annu. Rev. Entomol.* 43, 17–37. doi:10.1146/annurev.ento.43.1.17.

Doygun, H. (2009). Effects of urban sprawl on agricultural land: a case study of Kahramanmaras, Turkey. *Env. Monit Assess* 158, 471–478. doi:10.1007/s10661-008-0597-7.

Dubrovsky, M., Svoboda, M. D., Trnka, M., Hayes, M. J., Wilhite, D. A., Zalud, Z., et al. (2009). Application of relative drought indices in assessing climate-change impacts on drought conditions in Czechia. *Theor. Appl. Climatol.* 96, 155–171. doi:10.1007/s00704-008-0020-x.

Duran, M., and Akyildiz, G. K. (2011). Evaluating benthic macroinvertebrate fauna and water quality of Suleymanli Lake (Buldan-Denizli) in Turkey. *Acta zool bulg* 63, 169–178.

Ehleringer, J. R., and Björkman, O. (1978). Pubescence and leaf spectral characteristics in a desert shrub, *Encelia farinosa* . *Oecologia* 36, 151–162. doi:10.1007/BF00349805.

Elbakidze, L., Lu, L., and Eigenbrode, S. (2011). Evaluating vector-virus-yield interactions for peas and lentils under climatic variability: A limited dependent variable analysis. *Source J. Agric. Resour. Econ. J. Agric. Resour. Econ.* 36. Available at: http://www.jstor.org/stable/23243521 [Accessed June 1, 2018].

Elden, T. C., and Lambert, L. (1992). Mechanisms of potato leafhopper resistance in soybean lines isogenic for pubescence type. *Crop Sci.* 32, 1187. doi:10.2135/cropsci1992.0011183X003200050026x.

Elmas, C. (1994). A sustainable neighborhood in Urfa, Turkey. *Ekistics* 61, 183–187. Available at: http://www.jstor.org/stable/43623172.

Ennis, W. B., Williamson, R. E., and Dorschner, K. P. (1952). Studies on spray retention by leaves of different plants. *Weeds* 1, 274. doi:10.2307/4040120.

Ertabaklar, H., Toz, S. O., Ozkan, A. T., Rastgeldi, S., Balcioglu, I. C., and Ozbel, Y. (2005). Serological and entomological survey in a zoonotic visceral leishmaniasis focus of North Central Anatolia, Turkey: Corum province. *Acta Trop.* 93, 239–246. doi:10.1016/j.actatropica.2005.01.002.

FAO (2017). Exports of top 5 exporters of Lentils. *FAOSTAT - Crop. Livest. Prod.* Available at: http://www.fao.org/faostat/en/#data/TP/visualize [Accessed February 23, 2020].

FAO (2018). Production quantities of Lentils by country. *FAOSTAT - Crop.* Available at: http://www.fao.org/faostat/en/#data/QC [Accessed February 23, 2020].

Faour, G., Meslmani, Y., and Fayad, A. (2010). Climate change atlas of Syria. doi:10.13140/RG.2.2.26562.17601.

Febvay, G., Bonnin, J., Rahbé, Y., Bournoville, R., Delrot, S., and Bonnemain, J. L. (1988). Resistance of different lucerne cultivars to the pea aphid *Acyrthosiphon pisum*: influence of phloem composition on aphid fecundity. *Entomol. Exp. Appl.* 48, 127–134. doi:10.1111/j.1570-7458.1988.tb01155.x.

Fedoruk, L. K., Johnson, E. N., and Shirtliffe, S. J. (2011). The critical period of weed control for lentil in Western Canada. *Weed Sci.* 59, 517–526. doi:10.1614/WS-D-11-00051.1.

Fedoruk, L. K., and Shirtliffe, S. J. (2011). Herbicide choice and timing for weed control in imidazolinone-resistant lentil. *Weed Technol.* 25, 620–625. doi:10.1614/wt-d-11-00079.1.

Ferguson, M. E., Maxted, N., Slageren, M. Van, and Robertson, L. D. (2000). A re-assessment of the taxonomy of *Lens* Mill. (Leguminosae, Papilionoideae, Vicieae). *Bot. J. Linn. Soc.* 133, 41–59. doi:10.1111/j.1095-8339.2000.tb01536.x.

Fiala, J. V., Tullu, A., Banniza, S., Ginette, S. S., and Vandenberg, A. (2009). Interspecies transfer of resistance to anthracnose in lentil (*Lens culinaris* Medic.). *Crop Sci.* 49, 825–830. doi:10.2135/cropsci2008.05.0260.

Fox, J., and Weisberg, S. (2019). *An R companion to applied regression*. Third. Thousand Oaks, CA: SAGE PublicationsSage UK: London, England.

Fürstenberg-Hägg, J., Zagrobelny, M., and Bak, S. (2013). Plant defense against insect herbivores. *Int. J. Mol. Sci.* 14, 10242–97. doi:10.3390/ijms140510242.

Galán, C., Emberlin, J., Domínguez, E., Bryant, R. H., and Villamandos, F. (1995). A comparative analysis of daily variations in the Gramineae pollen counts at Córdoba, Spain and London. *Grana* 34, 189–198. doi:10.1080/00173139509429042.

Gao, L.-L., Klingler, J. P., Anderson, J. P., Edwards, O. R., and Singh, K. B. (2008). Characterization of pea aphid resistance in *Medicago truncatula*. *Plant Physiol.* 146, 996–1009. doi:10.1104/pp.107.111971.

Gavloski, J. (2017). Pea aphids on peas, fababeans and lentils. Carman Available at: https://www.gov.mb.ca/agriculture/crops/insects/aphids-on-peas.html [Accessed June 1, 2018].

Gerald Young, B. (2003). "Herbicide Adjuvants," in *Encyclopedia of Agrochemicals* (Hoboken, NJ, USA: John Wiley & Sons, Inc.). doi:10.1002/047126363X.agr007.

Gianoli, E., and González-Teuber, M. (2005). Environmental heterogeneity and population differentiation in plasticity to drought in *Convolvulus chilensis* (Convolvulaceae). *Evol. Ecol.* 19, 603–613. doi:10.1007/s10682-005-2220-5.

Giraudoux, P. (2018). pgirmess: Spatial analysis and data mining for field ecologists. Available at: https://cran.r-project.org/package=pgirmess.

Gitsopoulos, T. K., Damalas, C. A., and Georgoulas, I. (2014). Improving diquat efficacy on grasses by adding adjuvants to the spray solution before use. *Planta Daninha* 32, 355–360. doi:10.1590/S0100-83582014000200013.

Goffreda, J. C., Mutschler, M. A., Avé, D. A., Tingey, W. M., and Steffens, J. C. (1989). Aphid deterrence by glucose esters in glandular trichome exudate of the wild tomato, *Lycopersicon pennellii*. *J. Chem. Ecol.* 15, 2135–2147. doi:10.1007/BF01207444.

Gonzáles, W. L., Negritto, M. A., Suárez, L. H., and Gianoli, E. (2008). Induction of glandular and non-glandular trichomes by damage in leaves of *Madia sativa* under contrasting water regimes. *Acta Oecologica* 33, 128–132. doi:10.1016/j.actao.2007.10.004.

Gorim, L. Y., and Vandenberg, A. (2017). Evaluation of wild lentil species as genetic resources to improve drought tolerance in cultivated lentil. *Front. Plant Sci.* 8, 1129. doi:10.3389/fpls.2017.01129.

Gorim, L. Y., and Vandenberg, A. (2018). Can wild lentil genotypes help improve water use and transpiration efficiency in cultivated lentil? *Plant Genet. Resour. Characterisation Util.* 16, 459–468. doi:10.1017/S1479262117000399.

Gossen, B. D., Peng, G., Wolf, T. M., and McDonald, M. R. (2008). Improving spray retention to enhance the efficacy of foliar-applied disease- and pest-management products in field and row crops. *Can. J. Plant Pathol.* 30, 505–516. doi:10.1080/07060660809507550.

Government of Canada (2008). From impacts to adaptation: Canada in a changing climate 2007. , eds. D. S. Lemmen, F. J. Warren, J. Lacroix, and E. Bush Ottawa, ON Available at: http://adaptation2007.nrcan.gc.ca [Accessed September 27, 2017].

Gracia, J., Río, L. Del, Alonso, C., Benavente, J., and Anfuso, G. (2006). Historical evolution and present state of the coastal dune systems in the Atlantic coast of Cádiz (SW Spain): Palaeoclimatic and environmental implications. *J. Coast. Res.*, 55–63. Available at: http://www.jstor.org/stable/25737382.

Graham, P. H., and Vance, C. P. (2003). Legumes: importance and constraints to greater use. *Plant Physiol.* 131, 872–7. doi:10.1104/pp.017004.

Guerfel, M., Baccouri, O., Boujnah, D., Chaïbi, W., and Zarrouk, M. (2009). Impacts of water stress on gas exchange, water relations, chlorophyll content and leaf structure in

the two main Tunisian olive (*Olea europaea* L.) cultivars. *Sci. Hortic. (Amsterdam).* 119, 257–263. doi:10.1016/J.SCIENTA.2008.08.006.

Gulati, A., Schryer, P., and McHughen, A. (2002). Production of fertile transgenic lentil (*Lens culinaris* Medik) plants using particle bombardment. *Vitr. Cell. Dev. Biol. - Plant* 38, 316–324. doi:10.1079/IVP2002303.

Guo, H., Sun, Y., Peng, X., Wang, Q., Harris, M., and Ge, F. (2015). Up-regulation of abscisic acid signaling pathway facilitates aphid xylem absorption and osmoregulation under drought stress. *J. Exp. Bot.* 67, 681–693. doi:10.1093/jxb/erv481.

Gupta, D., and Sharma, S. K. (2006). Evaluation of wild *Lens* taxa for agro-morphological traits, fungal diseases and moisture stress in North Western Indian Hills. *Genet. Resour. Crop Evol.* 53, 1233–1241. doi:10.1007/s10722-005-2932-y.

Güvensen, A., ÇelIk, A., Topuz, B., and Öztürk, M. (2013). Analysis of airborne pollen grains in Denizli. *Turk. J. Botany* 37, 74–84. doi:10.3906/bot-1201-4.

Hanley, M. E., Lamont, B. B., Fairbanks, M. M., and Rafferty, C. M. (2007). Plant structural traits and their role in anti-herbivore defence. *Perspect. Plant Ecol. Evol. Syst.* 8, 157–178. doi:10.1016/J.PPEES.2007.01.001.

Harbour, J. D., Messersmith, C. G., and Ramsdale, B. K. (2003). Surfactants affect herbicides on kochia (*Kochia scoparia*) and Russian thistle (*Salsola iberica*). *Weed Sci.* 51, 430–434. doi:10.1614/0043-1745(2003)051[0430:sahokk]2.0.co;2.

Heap, I. (2020). The international survey of herbicide resistant weeds. Available at: www.weedscience.org [Accessed February 18, 2020].

Hess, F. D., Bayer, D. E., and Falk, R. H. (1974). Herbicide dispersal patterns: I. As a function of leaf surface. *Weed Sci.* 22, 394–401. Available at: http://www.jstor.org/stable/4042328.

Hess, F. D., and Falk, R. H. (1990). Herbicide deposition on leaf surfaces. *Weed Sci.* 38, 280–288. doi:10.1017/s004317450005654x.

Holder, C. D. (2012). The relationship between leaf hydrophobicity, water droplet retention, and leaf angle of common species in a semi-arid region of the western United States. *Agric. For. Meteorol.* 152, 11–16. doi:10.1016/j.agrformet.2011.08.005.

Hopkins, J. W. (1938). Agricultural meteorology: Correlation of air temperatures in central and southern Alberta and Saskatchewan with latitude, longitude and altitude. *Can. J. Res.* 16c, 16–26. doi:10.1139/cjr38c-002.

Hothorn, T., Bretz, F., and Westfall, P. (2008). Simultaneous inference in general parametric models. *Biometrical J.* 50, 346–363.

Hu, G., Balangcod, T., and Xiang, C.-L. (2012). Trichome micromorphology of the Chinese-Himalayan genus *Colquhounia* (Lamiaceae), with emphasis on taxonomic implications. *Biologia (Bratisl).* 67. doi:10.2478/s11756-012-0076-z.

Huangfu, C., Song, X., and Qiang, S. (2009). Morphological disparities in the epidermal and anatomical features of the leaf among wild *Brassica juncea* populations. *Weed Biol. Manag.* 9, 234–242. doi:10.1111/j.1445-6664.2009.00344.x.

Huttunen, P., Kärkkäinen, K., Løe, G., Rautio, P., and Ågren, J. (2010). Leaf Trichome Production and Responses to Defoliation and Drought in *Arabidopsis lyrata* (Brassicaceae). *Ann. Bot. Fenn.* 47, 199–207. doi:10.5735/085.047.0304.

Jenicek, M., and Ledvinka, O. (2020). Importance of snowmelt contribution to seasonal runoff and summer low flows in Czechia. *Hydrol. Earth Syst. Sci. Discuss.*, 1–23.

Ju, J., Bai, H., Zheng, Y., Zhao, T., Fang, R., and Jiang, L. (2012). A multi-structural and multi-functional integrated fog collection system in cactus. *Nat. Commun.* 3, 1247. doi:10.1038/ncomms2253.

Karasartova, D., Gureser, A. S., Gokce, T., Celebi, B., Yapar, D., Keskin, A., et al. (2018). Bacterial and protozoal pathogens found in ticks collected from humans in Corum province of Turkey. *PLoS Negl. Trop. Dis.* 12, e0006395. doi:10.1371/journal.pntd.0006395.

Kattan, Z. (1997). Chemical and environmental isotope study of precipitation in Syria. *J. Arid Environ.* 35, 601–615.

Khan, Z. R., Ward, J. T., and Norris, D. M. (1986). Role of trichomes in soybean resistance to cabbage looper, *Trichoplusia ni*. *Entomol. Exp. Appl.* 42, 109–117. doi:10.1111/j.1570-7458.1986.tb01010.x.

Khazaei, H., Caron, C. T., Fedoruk, M., Diapari, M., Vandenberg, A., Coyne, C. J., et al. (2016). Genetic diversity of cultivated lentil (*Lens culinaris* Medik.) and its relation to the world's agro-ecological zones. *Front. Plant Sci.* 7, 1093. doi:10.3389/fpls.2016.01093.

Kniss, A. R., and Odero, D. C. (2013). Interaction between preemergence ethofumesate and postemergence glyphosate. *Weed Technol.* 27, 47–53. doi:10.1614/WT-D-12-00050.1.

Kordan, B., Stec, K., Słomiński, P., Laszczak-Dawid, A., Wróblewska-Kurdyk, A., and Gabryś, B. (2019). Antixenosis potential in pulses against the pea aphid (Hemiptera: Aphididae). *J. Econ. Entomol.* 112, 465–474. doi:10.1093/jee/toy349.

Krimmel, B. A., and Pearse, I. S. (2013). Sticky plant traps insects to enhance indirect defence. *Ecol. Lett.* 16, 219–224. doi:10.1111/ele.12032.

Kumari, J., Ahmad, R., Chandra, S., and Jha, G. K. (2009). Determination of morpholological attributes imparting resistance against aphids (*Aphis craccivora* Koch) in lentil (*Lens culinaris* Medik). *Arch. Phytopathol. Plant Prot.* 42, 52–57. doi:10.1080/03235400600940806.

Ladizinsky, G. (1979). The origin of lentil and its wild genepool. *Euphytica* 28, 179–187. doi:10.1007/BF00029189.

Ladizinsky, G. (1993). Lentil domestication: On the quality of evidence and arguments. *Econ. Bot.* 47, 60–64. doi:10.1007/BF02862206.

Lam, W.-K. F., and Pedigo, L. P. (2001). Effect of trichome density on soybean pod feeding by adult bean leaf beetles (Coleoptera: Chrysomelidae). *J. Econ. Entomol.* 94, 1459–1463. doi:10.1603/0022-0493-94.6.1459.

Laskar, R. A., Khan, S., Deb, C. R., Tomlekova, N., Wani, M. R., Raina, A., et al. (2019). "Lentil (*Lens culinaris* Medik.) Diversity, cytogenetics and breeding," in *Advances in Plant Breeding Strategies: Legumes* (Springer International Publishing), 319–369. doi:10.1007/978-3-030-23400-3_9.

Lenth, R. (2019). emmeans: Estimated marginal means, aka least-squares means. Available at: https://cran.r-project.org/package=emmeans.

Lusa, M. G., Cardoso, E. C., Machado, S. R., and Appezzato-da-Glória, B. (2014). Trichomes related to an unusual method of water retention and protection of the stem apex in an

arid zone perennial species. *AoB Plants* 7. doi:10.1093/aobpla/plu088.

Mangiafico, S. (2019). rcompanion: Functions to support extension education program evaluation. Available at: https://cran.r-project.org/package=rcompanion.

Manthey, F., Szelezniak, E., and Nalewaja, J. (2009). "Relationship between spray droplet spread and herbicide phytotoxicity," in *Pesticide Formulations and Application Systems* (ASTM International), 182-182–10. doi:10.1520/stp11350s.

Massinon, M., Boukhalfa, H., and Lebeau, F. (2014). The effect of surface orientation on spray retention. *Precis. Agric.* 15, 241–254. doi:10.1007/s11119-013-9345-2.

McDonald, G. K., Hollaway, K. L., and McMurray, L. (2007). Increasing plant density improves weed competition in lentil (*Lens culinaris*). *Aust. J. Exp. Agric.* 47, 48. doi:10.1071/EA05168.

McMurray, L. S., Preston, C., Vandenberg, A., Mao, D., Oldach, K. H., Meier, K. S., et al. (2019). Development of high levels of metribuzin tolerance in lentil. *Weed Sci.* 67, 83–90. doi:10.1017/wsc.2018.57.

Mcvean, R. I. K., and Dixon, A. F. G. (2001). The effect of plant drought-stress on populations of the pea aphid *Acyrthosiphon pisum*. *Ecol. Entomol.* 26, 440–443. doi:10.1046/j.1365-2311.2001.00341.x.

McWhorter, C. G., Paul, R. N., and Ouzts, J. C. (1995). Bicellular trichomes of johnsongrass (*Sorghum halepense*) leaves: Morphology, histochemistry, and function. *Weed Sci.* 43, 201–208. doi:10.1017/s0043174500081078.

Mehrparvar, M., Zytynska, S. E., and Weisser, W. W. (2013). Multiple cues for winged morph production in an aphid metacommunity. *PLoS One* 8, e58323. doi:10.1371/journal.pone.0058323.

Miklas, P. N., Kelly, J. D., Beebe, S. E., and Blair, M. W. (2006). Common bean breeding for resistance against biotic and abiotic stresses: From classical to MAS breeding. *Euphytica* 147, 105–131. doi:10.1007/s10681-006-4600-5.

Muro Castrejón, F., Cruz-Vázquez, C., Fernández-Ruvalcaba, M., Molina-Torres, J., Cruz, J. S., and Parra, M. R. (2003). Repellence of *Boophilus microplus* larvae in *Stylosanthes humilis* and *Stylosanthes hamata* plants. *Parasitol Latinoam* 58, 118–121. Available at: https://scielo.conicyt.cl/pdf/parasitol/v58n3-4/art05.pdf [Accessed May 31, 2018].

Mutani, G., and Marchetti, L. (2015). Experimental investigation on green roofs' thermal performance in Turin (Italy). *J. Civ. Eng. Archit. Res* 2, 449–461. Available at: http://www.blueap.eu/site/en/ [Accessed June 18, 2020].

Mwakutuya, E. (2006). Epidemiology of stemphylium blight on lentil (*Lens culinaris*) in Saskatchewan.

Nachappa, P., Culkin, C. T., Saya, P. M., Han, J., and Nalam, V. J. (2016). Water stress modulates soybean aphid performance, feeding behavior, and virus transmission in soybean. *Front. Plant Sci.* 7. doi:10.3389/fpls.2016.00552.

Nalam, V., Louis, J., and Shah, J. (2019). Plant defense against aphids, the pest extraordinaire. *Plant Sci.* 279, 96–107. doi:10.1016/j.plantsci.2018.04.027.

Nalewaja, J. D., Devilliers, B., and Matysiak, R. (1996). Surfactant and salt affect glyphosate retention and absorption. *Weed Res.* 36, 241–247. doi:10.1111/j.1365-3180.1996.tb01653.x.

Ng, J. C. K., and Perry, K. L. (2004). Transmission of plant viruses by aphid vectors. *Mol. Plant Pathol.* 5, 505–511. doi:10.1111/j.1364-3703.2004.00240.x.

Ning, P., Wang, J., Zhou, Y., Gao, L., Wang, J., and Gong, C. (2016). Adaptional evolution of trichome in *Caragana korshinskii* to natural drought stress on the Loess Plateau, China. *Ecol. Evol.* 6, 3786–3795. doi:10.1002/ece3.2157.

Nleya, T., Vandenberg, A., and Walley, F. L. (2004). "Lentil | Agronomy," in *Encyclopedia of Grain Science*, ed. C. B. T.-E. of G. S. Wrigley (Oxford: Elsevier), 150–157. doi:https://doi.org/10.1016/B0-12-765490-9/00079-3.

Norsworthy, J. K., and Grey, T. L. (2004). Addition of nonionic surfactant to glyphosate plus chlorimuron. *Weed Technol.* 18, 588–593. doi:10.1614/wt-03-096r1.

Nunes, C., Araújo, S. ., Silva, J. M., Fevereiro, P., and Silva, A. B. (2009). Photosynthesis light curves: a method for screening water deficit resistance in the model legume *Medicago truncatula*. *Ann. Appl. Biol.* 155, 321–332. doi:10.1111/j.1744-7348.2009.00341.x.

Ogawa, K., and Miura, T. (2014). Aphid polyphenisms: trans-generational developmental regulation through viviparity. *Front. Physiol.* 5, 1. doi:10.3389/fphys.2014.00001.

Oghiakhe, S., Jackai, L. E. N., Makanjuola, W. A., and Hodgson, C. J. (1992). Morphology, distribution, and the role of trichomes in cowpea (*Vigna unguiculata*) resistance to the legume pod borer, *Maruca testulalis* (Lepidoptera: Pyralidae). *Bull. Entomol. Res.* 82, 499. doi:10.1017/S0007485300042577.

Ogle, D. H., Wheeler, P., and Dinno, A. (2020). FSA: Fisheries Stock Analysis. Available at: https://github.com/droglenc/FSA.

Peng, G., Wolf, T. M., Byer, K. N., and Caldwell, B. (2005). Spray retention on green foxtail (*Setaria viridis*) and its effect on weed control efficacy by *Pyricularia setariae*. *Weed Technol.* 19, 86–93. doi:10.1614/wt-03-261r.

Permana, A. D., Johari, A., Putra, R. E., Sastrodihardjo, S., and Ahmad, I. (2012). The influence of trichome characters of soybean (*Glycine max* Merrill) on oviposition preference of soybean pod borer *Etiella zinckenella* Treitschke (Lepidoptera: Pyralidae) in Indonesia. *J. Entomol. Nematol.* 4, 15–21. doi:10.5897/JEN11.020.

Pinheiro, J., Bates, D., DebRoy, S., and Sarkar, D. (2019). nlme: Linear and nonlinear mixed effects models. Available at: https://cran.r-project.org/package=nlme.

Postel, S. L. (2000). Entering an Era of Water Scarcity: The Challenges Ahead. *Ecol. Appl.* 10, 941–948. doi:10.1890/1051-0761(2000)010[0941:EAEOWS]2.0.CO;2.

Pratt, S. (2011). EU rejects lentils for glyphosate residue. Available at: https://www.producer.com/2011/04/eu-rejects-lentils-for-glyphosate-residue/ [Accessed March 16, 2020].

Procópio, S. O., Ferreira, E. A., Silva, E. A. M., Silva, A. A., Rufino, R. J. N., and Santos, J. B. (2003). Leaf anatomical studies in weed species widely common in Brazil. III - *Galinsoga parviflora, Crotalaria incana, Conyza bonariensis* and *Ipomoea cairica*. *Planta Daninha* 21, 1–9. doi:10.1590/s0100-83582003000100001.

R Core Team (2019). R: A language and environment for statistical computing. Available at: https://www.r-project.org/.

Rabani, E. M. (2018). Effects of drought and growth media on lentil growth characteristics. Available at: https://harvest-

test.usask.ca/bitstream/handle/10388/8461/MOHSENZADEHRABANI-THESIS-2018.pdf?sequence=1&isAllowed=y [Accessed February 9, 2020].

Ramsdale, B. K., and Messersmith, C. G. (2002). Adjuvant and herbicide concentration in spray droplets influence phytotoxicity. *Weed Technol.* 16, 631–637. Available at: https://doi.org/10.1614/0890-037X(2002)016[0631:AAHCIS]2.0.CO.

Redden, B., Maxted, N., Furman, B., and Coyne, C. (2007). "Lens biodiversity," in *Lentil*, eds. S. S. Yadav, D. L. McNeil, and P. C. Stevenson (Dordrecht: Springer Netherlands), 11–22. doi:10.1007/978-1-4020-6313-8_2.

Reddy, K. N., Locke, M. A., and Howard, K. D. (1995). Bentazon spray retention, activity, and foliar washoff in weed species. *Weed Technol.* 9, 773–778. doi:10.1017/s0890037x00024192.

Reyes, M. L., Laughton, A. M., Parker, B. J., Wichmann, H., Fan, M., Sok, D., et al. (2019). The influence of symbiotic bacteria on reproductive strategies and wing polyphenism in pea aphids responding to stress. *J. Anim. Ecol.* 88, 601–611. doi:10.1111/1365-2656.12942.

Riechers, D. E., Wax, L. M., Liebl, R. A., and Bullock, D. G. (1995). Surfactant effects on glyphosate efficacy. *Weed Technol.* 9, 281–285. doi:10.1017/s0890037x00023356.

Ritz, C., Baty, F., Streibig, J. C., and Gerhard, D. (2015). Dose-response analysis using R. *PLoS One* 10. Available at: http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0146021.

Rizwan, M., Aslam, M., Asghar, M. J., Abbas, G., Shah, T. M., and Shimelis, H. (2017). Pre-breeding of lentil (*Lens culinaris* Medik.) for herbicide resistance through seed mutagenesis. *PLoS One* 12. doi:10.1371/journal.pone.0171846.

Roberts, J. J., and Foster, J. E. (1983). Effect of leaf pubescence in wheat on the bird cherry oat aphid (Homoptera: Aphidae). *J. Econ. Entomol.* 76, 1320–1322. doi:10.1093/jee/76.6.1320.

Sakai, W. S., and Sanford, W. G. (1980). Ultrastructure of the water-absorbing trichomes of pineapple (*Ananas comosus*, Bromeliaceae). *Ann. Bot.* 46, 7–11. doi:10.2307/42761341.

Sánchez-Rodríguez, E., Rubio-Wilhelmi, Mm., Cervilla, L. M., Blasco, B., Rios, J. J., Rosales, M. A., et al. (2010). Genotypic differences in some physiological parameters symptomatic for oxidative stress under moderate drought in tomato plants. *Plant Sci.* 178, 30–40. doi:10.1016/j.plantsci.2009.10.001.

Sánchez, F. J., Manzanares, M., de Andrés, E. F., Tenorio, J. L., and Ayerbe, L. (2001). Residual transpiration rate, epicuticular wax load and leaf colour of pea plants in drought conditions. Influence on harvest index and canopy temperature. *Eur. J. Agron.* 15, 57–70. doi:10.1016/S1161-0301(01)00094-6.

Sandhu, J. S., and Singh, S. (2007). "History and origin," in *Lentil*, eds. S. S. Yadav, D. L. McNeil, and P. C. Stevenson (Dordrecht: Springer Netherlands), 1–9. doi:10.1007/978-1-4020-6313-8_1.

Santos Tozin, L. R. D., de Melo Silva, S. C., and Rodrigues, T. M. (2016). Non-glandular trichomes in *Lamiaceae* and *Verbenaceae* species: morphological and histochemical features indicate more than physical protection. *New Zeal. J. Bot.* 54. doi:10.1080/0028825X.2016.1205107.

Sanyal, D., Bhowmik, P. C., and Reddy, K. N. (2006). Influence of leaf surface

micromorphology, wax content, and surfactant on primisulfuron droplet spread on barnyardgrass (Echinochloa crus-galli) and green foxtail (Setaria viridis). *Weed Sci.* 54, 627–633. doi:10.1614/WS-05-173R.1.

Saskatchewan Ministry of Agriculture (2020). *2020 Guide to Crop Protection*. Government of Saskatchewan.

Savé, R., Biel, C., and de Herralde, F. (2000). Leaf pubescence, water relations and chlorophyll fluorescence in two subspecies of *Lotus Creticus* L. *Biol. Plant.* 43, 239–244. doi:10.1023/A:1002704327076.

Schilmiller, A. L., Last, R. L., and Pichersky, E. (2008). Harnessing plant trichome biochemistry for the production of useful compounds. *Plant J.* 54, 702–711. doi:10.1111/j.1365-313X.2008.03432.x.

Seefeldt, S. S., Jensen, J. E., and Patrick Fuerst, E. (1995). Log-logistic analysis of herbicide dose-response relationships. *Weed Technol.* 9, 218–227. Available at: https://about.jstor.org/terms [Accessed November 28, 2019].

Seo, P. J., and Park, C.-M. (2011). Cuticular wax biosynthesis as a way of inducing drought resistance. *Plant Signal. Behav.* 6, 1043–1045. doi:10.4161/psb.6.7.15606.

Sessiz, A., Alp, A., and Gursoy, S. (2010). Conservation and conventional tillage methods on selected soil physical properties and corn (*Zea mays* L.) yield and quality under cropping system in Turkey. *Bulg. J. Agric. Sci.* 16, 597–608.

Shade, R. E., and Kitch, L. W. (1983). Pea aphid (Homoptera: Aphididae) biology on glandular-haired *Medicago* Species. *Environ. Entomol.* 12, 237–240. doi:10.1093/ee/12.1.237.

Shahwar, D., Bhat, T. M., Ansari, M. Y. K., Chaudhary, S., and Aslam, R. (2017). Health functional compounds of lentil (*Lens culinaris* Medik): A review. *Int. J. Food Prop.* 20, S1–S15. doi:10.1080/10942912.2017.1287192.

Sharma, H. C., Sujana, G., and Manohar Rao, D. (2009). Morphological and chemical components of resistance to pod borer, *Helicoverpa armigera* in wild relatives of pigeonpea. *Arthropod. Plant. Interact.* 3, 151–161. doi:10.1007/s11829-009-9068-5.

Shields, L. M. (1950). Leaf xeromorphy as related to physiological and structural influences. *Bot. Rev.* 16, 399–447. doi:10.1007/BF02869988.

Singh, M., Bisht, I. S., Kumar, S., Dutta, M., Bansal, K. C., Karale, M., et al. (2014). Global wild annual *Lens* collection: A potential resource for lentil genetic base broadening and yield enhancement. *PLoS One* 9. doi:10.1371/journal.pone.0107781.

Singh, M., Orsenigo, J. R., and Shah, D. O. (1984). Surface tension and contact angle of herbicide solutions affected by surfactants. *J. Am. Oil Chem. Soc.* 61, 596–600. doi:10.1007/BF02677040.

Singh, M., Rana, M. K., Kumar, K., Bisht, I. S., Dutta, M., Gautam, N. K., et al. (2013). Broadening the genetic base of lentil cultivars through inter-sub-specific and interspecific crosses of *Lens* taxa. *Plant Breed.* 132, 667–675. doi:10.1111/pbr.12089.

Singh, S., and Singh, M. (2005). Evaluation of some adjuvants for improving glyphosate efficacy. *J. ASTM Int.* 2, 12158. doi:10.1520/jai12158.

Smitchger, J. A., Burke, I. C., and Yenish, J. P. (2012). The critical period of weed control in lentil (*Lens culinaris*) in the Pacific Northwest. *Weed Sci.* 60, 81–85. doi:10.1614/ws-d-11-00069.1.

Smith, C. M., and Chuang, W.-P. (2014). Plant resistance to aphid feeding: behavioral, physiological, genetic and molecular cues regulate aphid host selection and feeding. *Pest Manag. Sci.* 70, 528–540. doi:10.1002/ps.3689.

Sorensen, J. T. (2009). "Aphids," in *Encyclopedia of Insects* (Elsevier), 27–31. doi:10.1016/B978-0-12-374144-8.00008-4.

Statistics Canada (2019). Table 32-10-0015-01. *Farm supply Dispos. grains (x 1,000)*. doi:https://doi.org/10.25318/3210001501-eng.

Stefaniak, T. R., and McPhee, K. E. (2015). "Lentil," in *Handbook of Plant Breeding, vol 10*, ed. A. M. De Ron (New York, NY: Springer New York), 111–140. doi:10.1007/978-1-4939-2797-5_4.

Stenglein, S. A., Arambarri, A. M., Vizgarra, O. N., and Balatti, P. A. (2004). Micromorphological variability of leaf epidermis in Mesoamerican common bean (*Phaseolus vulgaris*, Leguminosae). *Aust. J. Bot.* 52, 73–80. doi:10.1071/BT02083.

Sun, Y., Guo, H., and Ge, F. (2016). Plant–aphid interactions under elevated CO_2: Some cues from aphid feeding behavior. *Front. Plant Sci.* 7, 502. doi:10.3389/fpls.2016.00502.

Sutherst, R. W., Jones, R. J., and Schnitzerling, H. J. (1982). Tropical legumes of the genus *Stylosanthes* immobilize and kill cattle ticks. *Nature* 295, 320–321. doi:10.1038/295320a0.

Suzuki, T. (2013). Spatial composition of intermountain settlements on slopes with high wintertime precipitation in Eastern and Southeastern Anatolia of Turkey: Case studies of Çevre village in Bitlis and Aran village in Mardin. *Intercult. Underst.* 3, 33–40. Available at: http://www.geomapapp.org [Accessed June 17, 2020].

Temple, R. E., and Hilton, H. W. (1963). The effect of surfactants on the water solubility of herbicides, and the foliar phytotoxicity of surfactants. *Weeds* 11, 297. doi:10.2307/4040537.

Terletskaya, N., and Kurmanbayeva, M. (2017). Change in leaf anatomical parameters of seedlings of different wheat species under conditions of drought and salt stress.

Tominack, R. L., and Tominack, R. (2000). Herbicide formulations. *J. Toxicol. Clin. Toxicol.* 38, 129–135. doi:10.1081/CLT-100100927.

Tullu, A., Diederichsen, A., Suvorova, G., and Vandenberg, A. (2011). Genetic and genomic resources of lentil: Status, use and prospects. *Plant Genet. Resour. Characterisation Util.* 9, 19–29. doi:10.1017/S1479262110000353.

van Zeist, W., Timmers, R. W., and Bottema, S. (1970). Studies of modern and Holocene pollen precipitation in southeastern Turkey. *Palaeohistoria*, 19–39.

Vandenberg, A., Banniza, S., Warkentin, T. D., Ife, S., Barlow, B., McHale, S., et al. (2006). CDC Redberry lentil. *Can. J. Plant Sci.* 86, 497–498. doi:10.4141/P05-071.

War, A. R., Paulraj, M. G., Ahmad, T., Buhroo, A. A., Hussain, B., Ignacimuthu, S., et al. (2012). Mechanisms of plant defense against insect herbivores. *Plant Signal. Behav.* 7, 1306–20. doi:10.4161/psb.21663.

Warne, T., Ahmed, S., Byker Shanks, C., and Miller, P. (2019). Sustainability dimensions of a North American lentil system in a changing world. *Front. Sustain. Food Syst.* 3, 88. doi:10.3389/fsufs.2019.00088.

Webster, J. A., Inayatullah, C., Hamissou, M., and Mirkes, K. A. (1994). Leaf pubescence

effects in wheat on yellow sugarcane aphids and greenbugs (Homoptera: Aphididae). *J. Econ. Entomol.* 87, 231–240. doi:10.1093/jee/87.1.231.

Werker, E. (2000). "Trichome diversity and development," in *Advances in botanical research* (Elsevier Ltd.), 1–35. doi:10.1016/S0065-2296(00)31005-9.

Wheeler, A. G., and Krimmel, B. A. (2015). Mirid (Hemiptera: Heteroptera) specialists of sticky plants: adaptations, interactions, and ecological implications. *Annu. Rev. Entomol.* 60, 393–414. doi:10.1146/annurev-ento-010814-020932.

Wickham, H. (2016). *ggplot2: Elegant Graphics for Data Analysis*. New York, NY: Springer-Verlag New York Available at: https://ggplot2.tidyverse.org.

Will, T., Furch, A. C. U., and Zimmermann, M. R. (2013). How phloem-feeding insects face the challenge of phloem-located defenses. *Front. Plant Sci.* 4, 336. doi:10.3389/fpls.2013.00336.

Wong, M. M. L., Gujaria-Verma, N., Ramsay, L., Yuan, H. Y., Caron, C., Diapari, M., et al. (2015). Classification and characterization of species within the genus *Lens* using genotyping-by-sequencing (GBS). *PLoS One* 10. doi:10.1371/journal.pone.0122025.

Wyrill, J. B. I., and Burnside, O. C. (1976). Absorption, translocation, and metabolism of 2,4-D and glyphosate in common milkweed and hemp dogbane. *Weed Sci.* 24, 557–566. Available at: http://www.jstor.org/stable/4042607.

Xie, H. S., Hsiao, A. I., and Quick, W. A. (1997). Influence of drought on graminicide phytotoxicity in wild oat (*Avena fatua*) grown under different temperature and humidity conditions. *J. Plant Growth Regul.* 16, 233–237. doi:10.1007/PL00007002.

Xing, Z., Liu, Y., Cai, W., Huang, X., Wu, S., and Lei, Z. (2017). Efficiency of trichome-based plant defense in *Phaseolus vulgaris* depends on insect behavior, plant ontogeny, and structure. *Front. Plant Sci.* 8, 2006. doi:10.3389/fpls.2017.02006.

Xu, J., Smith, S., Smith, G., Wang, W., and Li, Y. (2019). Glyphosate contamination in grains and foods: An overview. *Food Control* 106, 106710. doi:https://doi.org/10.1016/j.foodcont.2019.106710.

Xu, L., and Baldocchi, D. D. (2003). Seasonal trends in photosynthetic parameters and stomatal conductance of blue oak (*Quercus douglasii*) under prolonged summer drought and high temperature. *Tree Physiol.* 23, 865–877. doi:10.1093/treephys/23.13.865.

Xu, L., Zhu, H., Ozkan, H. E., Bagley, W. E., and Krause, C. R. (2011). Droplet evaporation and spread on waxy and hairy leaves associated with type and concentration of adjuvants. *Pest Manag. Sci.* 67, 842–851. doi:10.1002/ps.2122.

Yildizdoğan, Z., İkten, C., Mutlu, N., and Toker, C. (2016). Genetic relationships among the genera *Cicer* L., *Lathyrus* L., *Lens* Mill., and *Vicia* L., together with similarity of *Lens* taxa based on morphological and AFLP markers. *Turk. J. Botany* 40, 566–575. doi:10.3906/bot-1507-9.

Yilmaz, Z. (2007). Evaluation of energy efficient design strategies for different climatic zones: Comparison of thermal performance of buildings in temperate-humid and hot-dry climate. *Energy Build.* 39, 306–316. doi:10.1016/j.enbuild.2006.08.004.

Yoshida, M., Cowgill, S. E., and Wightman, J. A. (1997). Roles of oxalic and malic acids in chickpea trichome exudate in host-plant resistance to *Helicoverpa armigera*. *J. Chem. Ecol.* 23, 1195–1210. doi:10.1023/B:JOEC.0000006395.45516.e8.

Yu, Y., Zhu, H., Frantz, J. M., Reding, M. E., Chan, K. C., and Ozkan, H. E. (2009).

Evaporation and coverage area of pesticide droplets on hairy and waxy leaves. *Biosyst. Eng.* 104, 324–334. doi:https://doi.org/10.1016/j.biosystemseng.2009.08.006.

Yürekli, K. (2015). Impact of climate variability on precipitation in the Upper Euphrates–Tigris Rivers Basin of Southeast Turkey. *Atmos. Res.* 154, 25–38. doi:10.1016/j.atmosres.2014.11.002.

Yusa, A., Berry, P., Cheng, J. J., Ogden, N., Bonsal, B., Stewart, R., et al. (2015). Climate change, drought and human health in Canada. *Int. J. Environ. Res. Public Health* 12, 8359–8412. doi:10.3390/ijerph120708359.

Zhang, T., Johnson, E. N., Mueller, T. C., and Willenborg, C. J. (2017). Early application of harvest aid herbicides adversely impacts lentil. *Agron. J.* 109, 239–248. doi:10.2134/agronj2016.07.0419.

Zhang, T., Johnson, E. N., and Willenborg, C. J. (2016a). Evaluation of harvest-aid herbicides as desiccants in lentil production. *Weed Technol.* 30, 629–638. doi:10.1614/wt-d-16-00007.1.

Zhang, Y., Wang, X.-X., Jing, X., Tian, H.-G., and Liu, T.-X. (2016b). Winged pea aphids can modify phototaxis in different development stages to assist their host distribution. *Front. Physiol.* 7, 307. doi:10.3389/fphys.2016.00307.

Zhou, J., Tao, B., Messersmith, C. G., and Nalewaja, J. D. (2007). Glyphosate efficacy on velvetleaf (*Abutilon theophrasti*) is affected by stress. *Weed Sci.* 55, 240–244. doi:10.1614/ws-06-173.1.

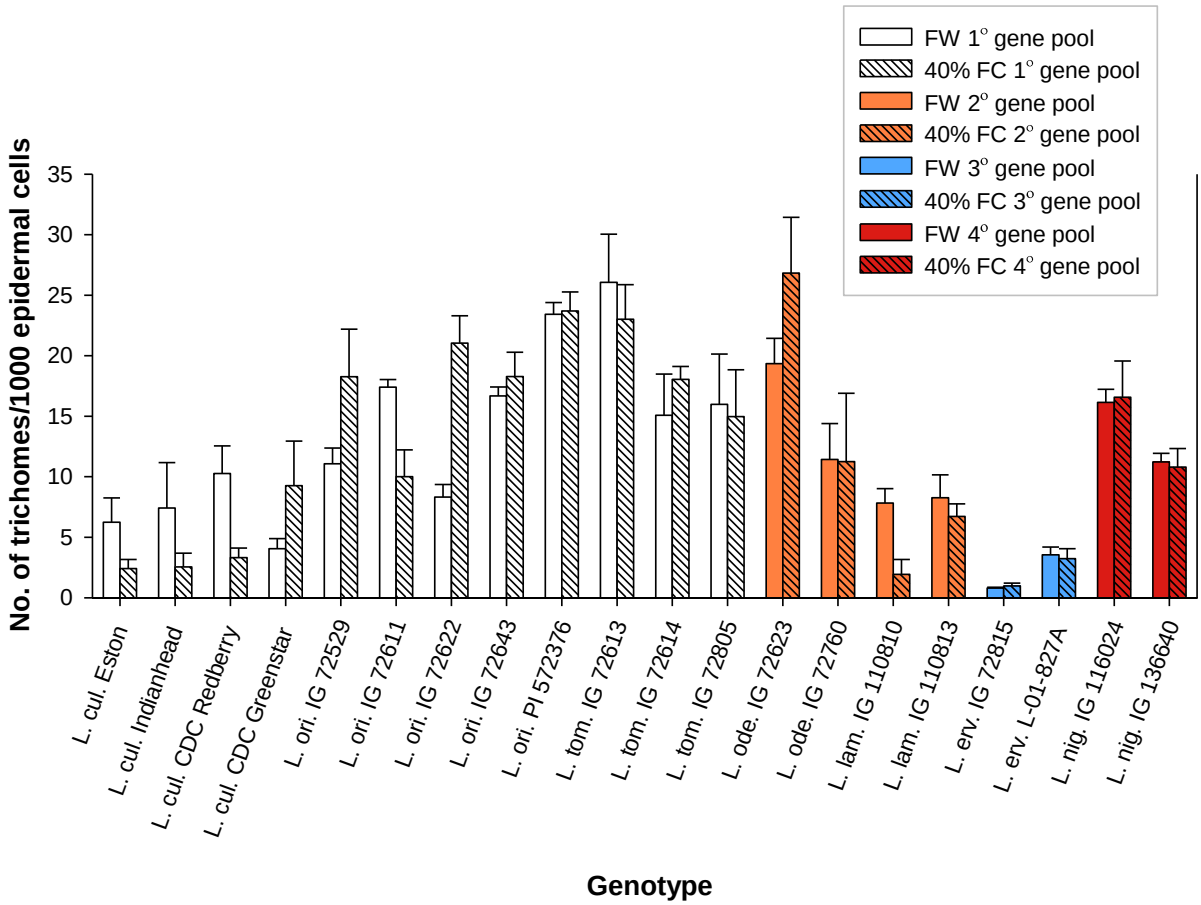
Zhu, H., and Lin, J. (2016). Coverage area and fading time of surfactant-amended herbicidal droplets on cucurbitaceous leaves. *Trans. ASABE* 59, 829–838. doi:10.13031/trans.59.11427.

Zohary, D. (1972). The wild progenitor and the place of origin of the cultivated lentil: *Lens culinaris*. *Econ. Bot.* 26, 326–332. Available at: http://www.jstor.org/stable/4253374.

Züst, T., and Agrawal, A. A. (2016). Mechanisms and evolution of plant resistance to aphids. *Nat. Plants* 2, 1–9. doi:10.1038/nplants.2015.206.

Appendices

Appendix 1 Trichome density per 1000 epidermal cells of adaxial leaflet surface of wild and cultivated lentil species grown under fully watered (FW) and 40% field capacity (40% FC) conditions. The error bars are standard errors of treatment means.



Appendix 2 ANOVA of trichome density of adaxial leaf surface of 20 genotypes of wild and cultivated lentil species grown under two treatments (FW and 40% FC conditions) under controlled conditions.

Source	Numerator df	Denominator df	F-value	p-value
Genotype	19	73	12.33	<0.0001
Treatment	1	73	2.2416	0.1387
Genotype x Treatment	19	73	2.1267	0.0115

Appendix 3 ANOVA of epidermal cell density/mm^2 of adaxial leaf surface of 20 genotypes of wild and cultivated lentil species grown under two treatments (FW and 40% FC conditions) under controlled conditions.

Source	Numerator df	Denominator df	F-value	p-value
Genotype	19	73	4.1803	<0.0001
Treatment	1	73	1.8785	0.1747
Genotype x Treatment	19	73	0.8614	0.6291

Appendix 4 ANOVA of stomatal index (%) of adaxial leaf surface of 20 genotypes of wild and cultivated lentil species grown under two treatments (FW and 40% FC conditions) under controlled conditions.

Source	Numerator df	Denominator df	F-value	p-value
Genotype	19	73	4.374	<0.0001
Treatment	1	73	0.524	0.4714
Genotype x Treatment	19	73	0.722	0.7849

Appendix 5 ANOVA of trichome length on adaxial leaf surface of 20 genotypes of wild and cultivated lentil species grown under two treatments (FW and 40% FC conditions) under controlled conditions.

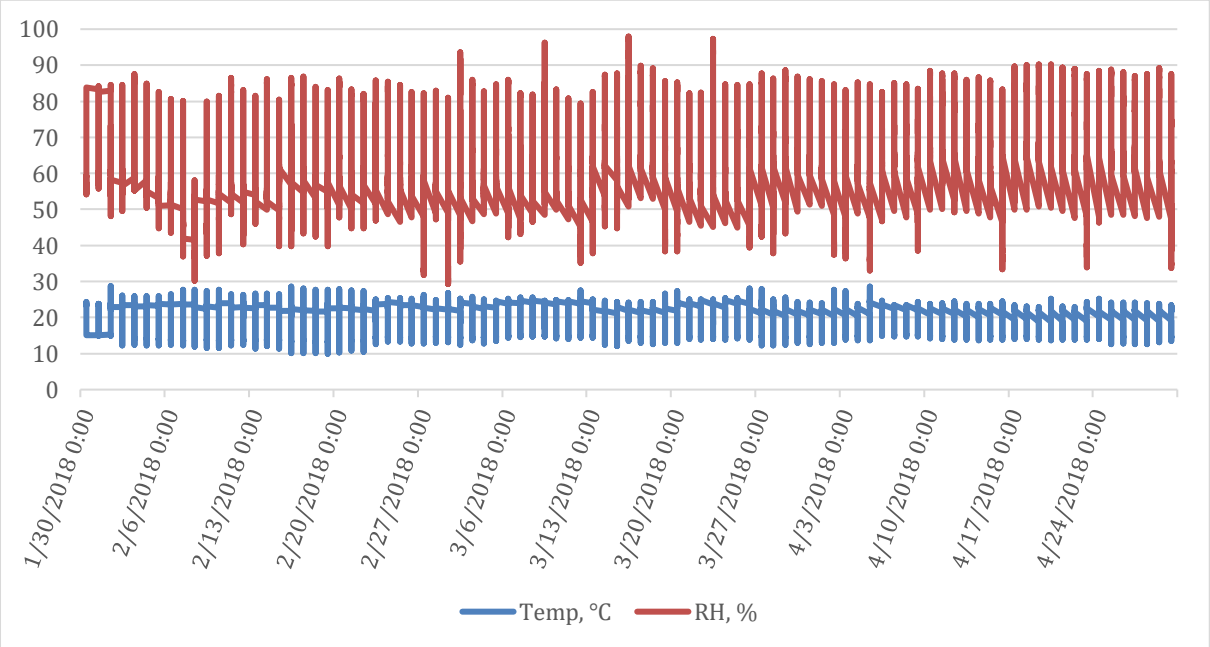
Source	Numerator df	Denominator df	F-value	p-value
Genotype	19	73	5.361	<0.0001
Treatment	1	73	1.458	0.2312
Genotype x Treatment	19	73	1.586	0.0831

Appendix 6 ANOVA of transpiration water loss of 12 genotypes of wild and cultivated lentil species grown under two treatments (FW and 40% FC conditions) under controlled conditions.

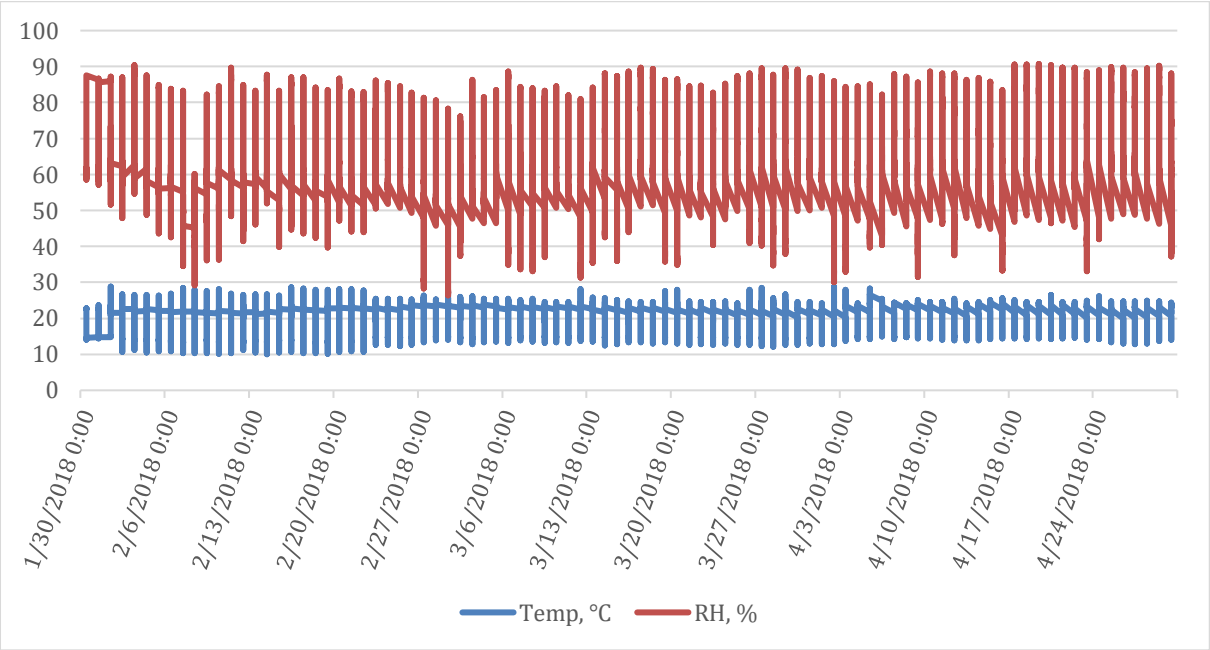
Source	Numerator df	Denominator df	F-value	p-value
Genotype	11	42	6.13827	<0.0001
Treatment	1	42	11.07838	0.0018
Genotype x Treatment	11	42	2.24594	0.0296

Appendix 7 Temperature and relative humidity under controlled settings in Phytotron facility at University of Saskatchewan while growing twenty genotypes within seven *Lens* spp. under FW and 40% FC condition (A) Data from first data logger (B) Data from second data logger (C) Data from third data logger (D) Data from fourth data logger

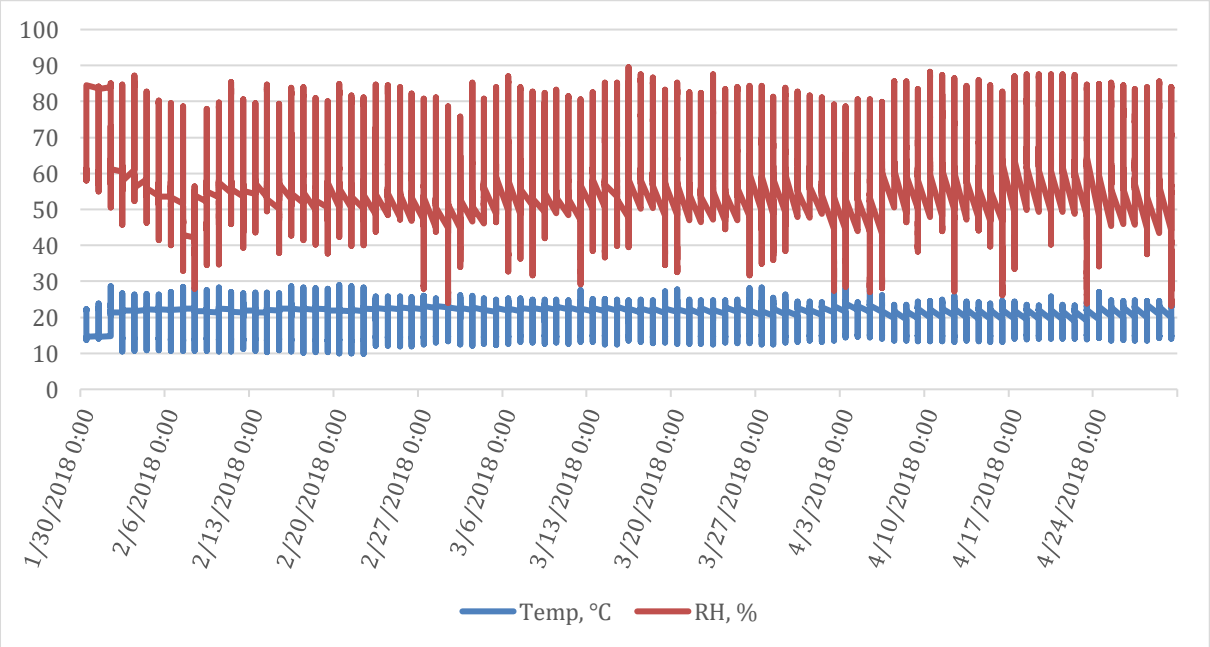
(A)



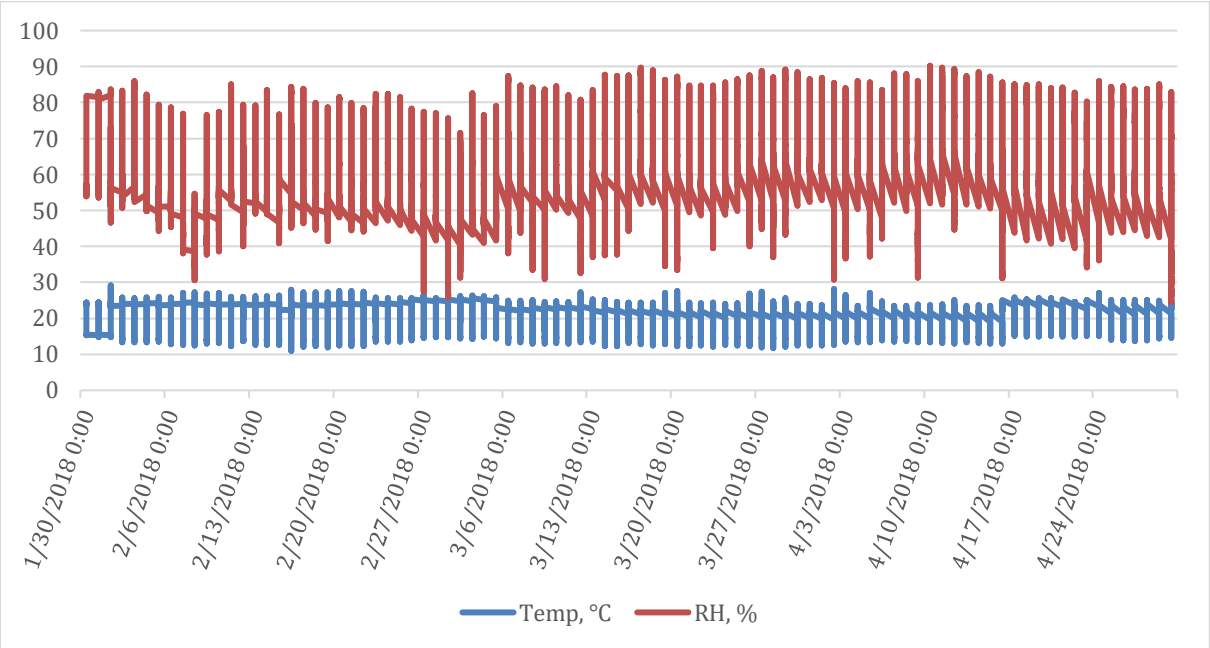
(B)


100
90
80
70
60
50
40
30
20
10
0
1/30/2018 0:00
2/6/2018 0:00
2/13/2018 0:00
2/20/2018 0:00
2/27/2018 0:00
3/6/2018 0:00
3/13/2018 0:00
3/20/2018 0:00
3/27/2018 0:00
4/3/2018 0:00
4/10/2018 0:00
4/17/2018 0:00
4/24/2018 0:00
Temp, °C
RH, %

(C)


100
90
80
70
60
50
40
30
20
10
0
1/30/2018 0:00
2/6/2018 0:00
2/13/2018 0:00
2/20/2018 0:00
2/27/2018 0:00
3/6/2018 0:00
3/13/2018 0:00
3/20/2018 0:00
3/27/2018 0:00
4/3/2018 0:00
4/10/2018 0:00
4/17/2018 0:00
4/24/2018 0:00
Temp, °C
RH, %

(D)



Appendix 8 ANOVA of shoot spray retention per unit area of *L. cul.* CDC Redberry and *L. tom.* IG 72805 after spraying with two treatments (Water and Water + non-ionic surfactant solutions).

Source	**df**	**Sum Sq**	**Mean Sq**	**F-value**	**Pr (>F)**
Treatment	1	0.01616	0.01616	2.894	0.0944
Genotype	1	0.00055	0.00055	0.098	0.7554
Treatment x Genotype	1	0.14658	0.14658	26.247	< 0.0001
Residuals	56	0.31272	0.00558		

Appendix 9 ANOVA of shoot spray retention per unit dry weight of *L. cul.* CDC Redberry and *L. tom.* IG 72805 after spraying with two treatments (Water and Water + non-ionic surfactant solutions).

Source	**df**	**Sum Sq**	**Mean Sq**	**F-value**	**Pr (>F)**
Treatment	1	972	972	4.581	0.0367
Genotype	1	6	6	0.03	0.8642
Treatment x Genotype	1	6239	6239	29.394	< 0.0001
Residuals	56	11887	212		

Appendix 10 ANOVA of shoot spray retention per unit area of NAM 38 sublines after spraying with two treatments (Water and Water + non-ionic surfactant solutions).

Source	df	Sum Sq	Mean Sq	F-value	Pr (>F)
Treatment	1	1.15	1.1497	14.81	0.000146
Genotype	11	6.547	0.5952	7.667	< 0.0001
Treatment x Genotype	11	3.95	0.3591	4.626	< 0.0001
Residuals	296	22.977	0.0776		

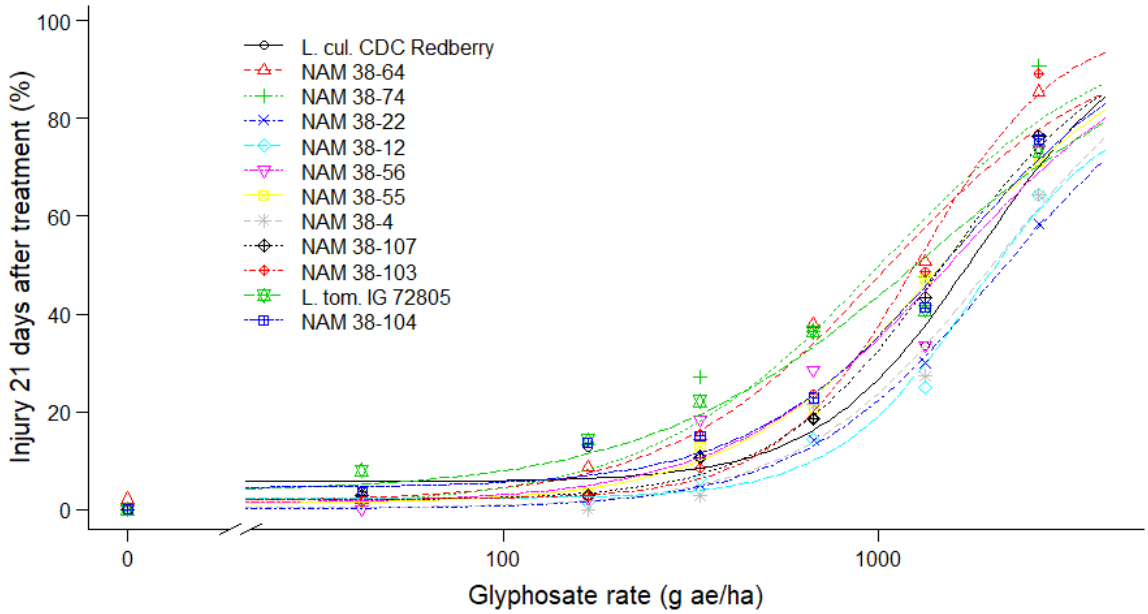
Appendix 11 ANOVA of shoot spray retention per unit area of NAM 38 sublines after spraying with two treatments (Water and Water + non-ionic surfactant solutions).

Source	df	Sum Sq	Mean Sq	F-value	Pr (>F)
Treatment	1	106210	106210	44.875	< 0.0001
Genotype	11	217046	19731	8.337	< 0.0001
Treatment x Genotype	11	121367	11033	4.662	< 0.0001
Residuals	296	700577			

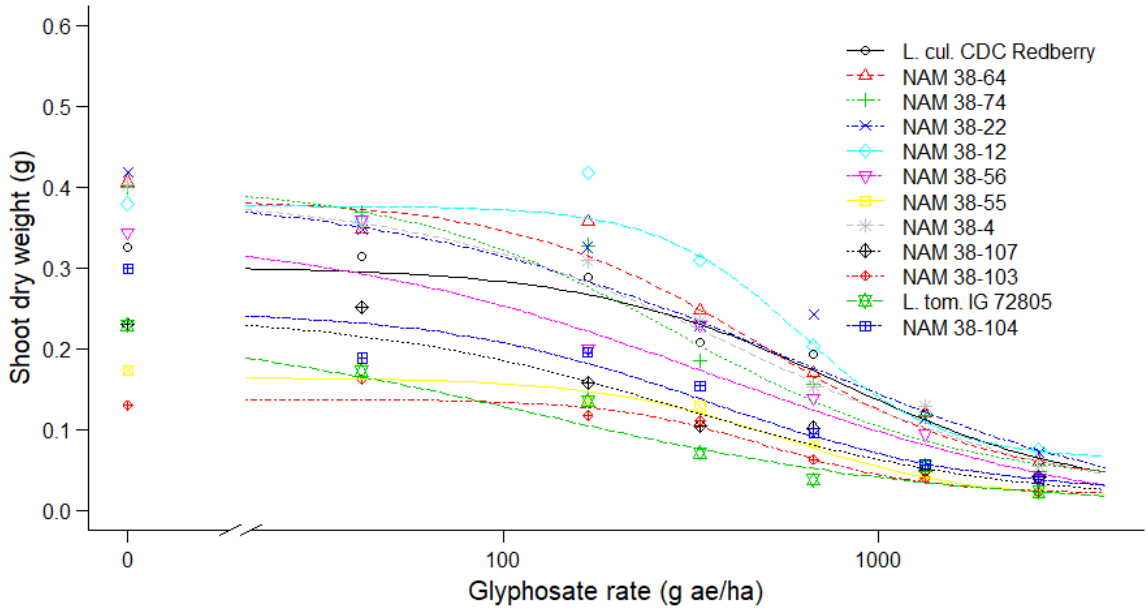
Appendix 12 ANOVA of shoot dry weight of 12 NAM 38 sublines after spraying with 8 glyphosate doses. Response variable was transformed with log transformation.

Source	df	Sum Sq	Mean Sq	F-value	Pr (>F)
Dose	7	424	60.58	264.557	< 0.0001
Genotype	11	101.2	9.2	40.198	< 0.0001
Dose x Genotype	77	20	0.26	1.133	0.217
Residuals	570	130.5	0.23		

Appendix 13 Injury at 21 days after spraying 12 NAM 38 sublines and parents with glyphosate doses ranging from 0-5340 g ae/ha. Genotypes in the legend are arranged in order of least to most trichome coverage on the leaf.



Appendix 14 Responses of 12 NAM 38 sublines and parents to Roundup PowerMax (glyphosate rate ranging from 0-5340 g ae/ha) expressed as a function of shoot dry mass 21 days after application. Genotypes in the legend are arranged in order of least to most trichome coverage on the leaf.



Appendix 15 Parameter estimates and standard errors (SE) from four-parameter log-logistic model of dry weight of 12 NAM 38 sublines and parents treated with glyphosate doses ranging from 0-5340 g ae/ha. b-slope, c-lower limit, d-upper limit, e-ED_{50} or dose eliciting 50% response. 12 NAM 38 sublines are ranked in the order of increasing trichome coverage (1 = least trichome coverage, 12 = most trichome coverage). Non-normality/heterogeneity in the model was adjusted through optimal Box-Cox transformation.

Rank	Genotype	b (SE)	c (SE)	d (SE)	e (SE)
1	*L. cul.* CDC Redberry	1.293 (0.476)	0.017 (0.027)	0.302 (0.029)	778.01 (208.047)
2	NAM 38-64	1.345 (0.373)	0.028 (0.018)	0.385 (0.035)	475.503 (108.898)
3	NAM 38-74	1.159 (0.298)	0.031 (0.017)	0.405 (0.039)	293.482 (76.853)
4	NAM 38-22	0.794 (0.321)	-0.023 (0.061)	0.396 (0.046)	594.314 (243.808)
5	NAM 38-12	2.305 (0.664)	0.063 (0.013)	0.377 (0.027)	613.061 (101.475)
6	NAM 38-56	0.796 (0.222)	-0.017 (0.029)	0.347 (0.038)	378.115 (134.165)
7	NAM 38-55	1.731 (0.639)	0.014 (0.007)	0.164 (0.018)	557.418 (144.777)
8	NAM 38-4	0.977 (0.303)	0.004 (0.03)	0.39 (0.041)	440.635 (130.253)
9	NAM 38-107	1.02 (0.281)	0.011 (0.013)	0.244 (0.028)	292.761 (96.617)
10	NAM 38-103	2.049 (0.79)	0.02 (0.006)	0.138 (0.015)	514.349 (128.658)
11	*L. tom.* IG 72805	0.813 (0.262)	0.005 (0.013)	0.229 (0.03)	128.503 (54.412)
12	NAM 38-104	1.221 (0.828)	0.02 (0.021)	0.247 (0.043)	353.762 (159.45)

Appendix 16 Test statistics and p-values of parameters from four-parameter log-logistic model of dry weight of 12 NAM 38 sublines treated with glyphosate doses ranging from 0-5340 g ae/ha. b-slope, c-lower limit, d-upper limit, e-ED_{50} or dose eliciting 50% response. Data were transformed using Box-Cox transformation.

	b		**c**		**d**		**e**	
	t-value	**p-value**	**t-value**	**p-value**	**t-value**	**p-value**	**t-value**	**p-value**
L. cul. CDC Redberry	2.716	0.00678	0.617	0.53738	10.281	< 0.0001	3.740	0.00020
NAM 38-64	3.605	0.00034	1.566	0.11789	11.155	< 0.0001	4.367	< 0.0001
NAM 38-74	3.883	0.00011	1.779	0.07580	10.468	< 0.0001	3.819	0.00015
NAM 38-22	2.473	0.01365	-0.377	0.70640	8.522	< 0.0001	2.438	0.01506
NAM 38-12	3.469	0.00056	4.883	< 0.0001	14.044	< 0.0001	6.041	< 0.0001
NAM 38-56	3.584	0.00036	-0.592	0.55437	9.054	< 0.0001	2.818	0.00498
NAM 38-55	2.707	0.00697	1.919	0.05539	8.989	< 0.0001	3.850	0.00013
NAM 38-4	3.227	0.00132	0.150	0.88075	9.636	< 0.0001	3.383	0.00076
NAM 38-107	3.629	0.00031	0.804	0.42173	8.801	< 0.0001	3.030	0.00255
NAM 38-103	2.593	0.00974	3.417	0.00067	9.325	< 0.0001	3.998	< 0.0001
L. tom. IG 72805	3.096	0.00205	0.412	0.68082	7.581	< 0.0001	2.362	0.01850
NAM 38-104	1.474	0.14087	0.955	0.33974	5.747	< 0.0001	2.219	0.02687

Appendix 17 Multiple comparison test (Z-value (p-value)) of aphid mortality in adults and nymphs after Kruskal-Wallis test using Dunn test and adjusting p-values using Bonferroni method.

	***L. cul.* CDC Maxim Adult**	***L. cul.* CDC Redberry Adult**	***L. tom.* IG 72805 Adult**	***L. cul.* CDC Maxim Nymph**	***L. cul.* CDC Redberry Nymph**
***L. cul.* CDC Redberry Adult**	0.757 (1.000)				
***L. tom.* IG 72805 Adult**	2.970 (0.0446)	2.152 (0.4703)			
***L. cul.* CDC Maxim Nymph**	-1.945 (0.7770)	-2.663 (0.1162)	-4.914 (<0.0001)		
***L. cul.* CDC Redberry Nymph**	-0.6528 (1.000)	-1.383 (1.000)	-3.563 (0.0055)	1.253 (1.000)	
***L. tom.* IG 72805 Nymph**	1.241 (1.000)	0.459 (1.000)	-1.729 (1.000)	3.186 (0.0216)	1.869 (0.9242)

Appendix 18 Multiple comparison test (Z-value (p-value)) of aphid maturity time on three genotypes after Kruskal-Wallis test using Dunn test and adjusting p-values using Bonferroni method.

	***L. cul.* CDC Maxim**	***L. cul.* CDC Redberry**
***L. cul.* CDC Redberry**	3.729 (0.0006)	
***L. tom.* IG 72805**	2.793 (0.0156)	-0.993 (0.9626)

Appendix 19 Pairwise comparison (Z ratio (p-value)) of least-squares means of maximum adult size reached by pea aphids after rearing on three genotypes using Tukey method and fitting GLM.

	***L. cul.* CDC Maxim**	***L. cul.* CDC Redberry**
***L. cul.* CDC Redberry**	-1.554 (0.2657)	
***L. tom.* IG 72805**	-3.592 (0.0010)	-1.957 (0.1229)

Appendix 20 Multiple comparison (Z-value (p-value)) of total winged aphids on three genotypes after Kruskal-Wallis test using Dunn test and adjusting p-values using Bonferroni method.

	***L. cul.* CDC Maxim**	***L. cul.* CDC Redberry**
***L. cul.* CDC Redberry**	-0.476 (1.000)	
***L. tom.* IG 72805**	1.1085 (0.8029)	1.5621 (0.3548)